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Influence of Diet and Feed Restriction on Kidney Function in Aging Male Rats

Sarah May Tucker
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Sarah May Tucker entitled "Influence of Diet and Feed Restriction on Kidney Function in Aging Male Rats." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Nutrition.

Roy E. Beauchene, Major Professor

We have read this dissertation and recommend its acceptance:

Jane R. Savage, Rossie L. Mason, Grayce Goertz, Mary Rose Gram

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

July 31, 1974

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Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

INFLUENCE OF DIET AND FEED RESTRICTION ON KIDNEY FUNCTION IN AGING MALE RATS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Sarah May Tucker

August 1974

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ABSTRACT

The influence of diet and feed restriction on kidney function was studied in aging male albino rats. Rats were fed either a commercial feed (LB) or a modified human diet (MHD) from weaning until sacrifice at either 12 or 24 months of age. Animals sacrificed at 12 months of age were either restricted or fed LB ad libitum from 1 month of age until sacrifice. Animals fed LB and sacrificed at 24 months of age were restricted for one of the following intervals: A, no restriction; R, restriction from 1 month to 24 months; RA, restriction from 1 month to 12 months; AR, restriction from 12 to 24 months. Animals eating MHD were either restricted or ad libitum-fed from 1 month to 24 months of age. Restriction was accomplished by making feed available for only 15 out of each 48 hours and resulted in terminal body weights of group R which were 66% as great as those of group A. Terminal body weights of groups AR and RA were 84 and 87%, respectively, those of group A.

Calculated growth rate (k) based on first year data was slower for animals fed a restricted level of LB as compared to those fed ad libitum. However, there was no difference in growth rates of restricted and ad libitum-fed animals eating MHD, and the rate for these animals was not different from that for rats eating a restricted level of LB.

Biochemical parameters considered to be measures of kidney function and used in this study included tests for urinary protein, creatinine and osmolarity, blood urea nitrogen (BUN) and serum creatinine, in vitro transport of paraaminohippuric acid (PAH) by kidney slices and sodium-potassium-activated-adenosine triphosphatase (Na-K-ATPase) activity of kidney microsomes. Kidneys were also examined microscopically for

lesions which might be expected to impair kidney function.

Results showed an absence of kidney lesions in animals restricted from 1 month of age, and only 1 rat (8%) in the group fed ad libitum to 12 months before restriction was begun (AR) showed evidence of inflammation, and this was only moderate. In contrast, 50% of rats fed MHD ad libitum and 67% of those similarly fed LB had moderate to severe lesions by the time they were sacrificed at 24 months. The magnitude of proteinuria was positively correlated with lesion scores for ad libitum-fed rats.

Urine volume increased with age only for ad libitum-fed rats eating MHD but tended to do so for those similarly fed LB. Urinary creatinine/body weight ratio was higher for groups eating MHD than for those eating LB. Urinary creatinine/body weight ratio decreased more between 15 and 21 months of age for rats fed ad libitum throughout the study and for those of group RA than for animals restricted throughout or those of group AR. Urinary protein increased much more with age of ad libitum-fed than of restricted (at any time) animals eating either diet. Urine osmolarity was complicated by influence of feeding pattern as well as by kidney performance.

Serum creatinine and BUN levels were influenced more by type of diet than by feeding patterns with the former parameter being higher for animals eating MHD than for those eating LB, and the latter, lower. There was a tendency toward higher BUN levels for ad libitum-fed than for restricted rats eating LB.

Total PAH transport was higher by kidney slices from young than from old rats, by restricted (at any time) than by ad libitum-fed ones

and by those fed MHD than by those similarly fed LB. There were tendencies toward decreasing activity of Na-K-ATPase (as percent of total ATPase) with age and toward lower activity by microsomes from kidneys of rats eating MHD than by those eating LB. There was a slight tendency toward higher activity by restricted than ad libitum-fed animals of the same age eating LB.

In general, feed restriction during either the first, the second or both years of the life of a rat was beneficial in delaying age-associated changes in kidney function as measured by PAH transport, proteinuria and kidney lesions. BUN levels and serum and urinary creatinine levels tended to be influenced favorably by restriction. Most parameters were modified by diet as well as by restriction with kidney performance being generally improved in animals eating MHD as compared with those comparably fed LB. Differences in kidney function among treatment groups may have been related to growth rates as well as intakes of calories and/or protein.

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CHAPTER I

INTRODUCTION

Feed restriction has long been recognized as beneficial in extending the life spans of various species, including rats and mice, but little is known about what effects the restriction has on the actual physiological processes of aging.

The present investigation attempted to define the effects of feed restriction and diet on the kidney function of aging rats. Earlier studies have shown that in vitro transport by kidneys of old rats is not as efficient as is that by kidneys from young adult animals. Rats show an increase in the number of severe renal lesions associated with chronic nephrosis and glomerulonephritis with age, and feed restriction has been reported to delay the onset of these lesions. The basis of the present study was the belief that feed restriction should also be beneficial in slowing down the biochemical changes which indicate a decreased renal function of old rats.

It was hypothesized that restriction of feed intake beginning after weaning and continuing until rats were sacrificed at 2 years of age would result in improved renal function of such rats as compared with kidneys from ad libitum-fed rats of the same age. It was further hypothesized that it would not be necessary to restrict continuously but that similar improvement could be observed if rats were restricted from weaning to 1 year of age and that some improvement would occur even if restriction of feed intake were not begun until animals were 1 year of age if continued until sacrifice. The study was extended to propose

that restriction of a nutritionally adequate diet composed of foods humans normally eat would lead to changes similar to those observed when animals were given a restricted level of a commercial rat feed.

Biochemical parameters considered to be measures of kidney function and performed on urine, blood and kidneys of animals in the study included the following: urinary protein, creatinine and osmolarity; blood urea nitrogen and serum creatinine; in vitro transport of paraaminohippuric acid (PAH) by kidney slices; and sodium-potassium-activated adenosine triphosphatase activity of kidney microsomes. Kidneys were also examined microscopically for lesions which might be expected to impair kidney function.

An increase in urinary protein denotes a decrease in the ability of the kidney to prevent the loss of macromolecules from the body. A decrease in creatinine excretion indicates an inability of the kidneys to adequately clear creatinine from the blood and might be expected to be accompanied by an increase in serum creatinine. A rise in blood urea nitrogen is another indication of decreased renal clearance of waste products from the blood while measurement of urine osmolarity is an indication of renal concentrating ability. PAH transport by kidney slices is an in vitro measure of the ability of the kidney to do osmotic work. The activity of sodium-potassium-activated adenosine triphosphatase is believed to be related to the utilization of energy from ATP necessary for active transport.

In accordance with the stated hypothesis it was anticipated that blood urea nitrogen, serum creatinine and urinary protein would be lower in restricted animals than in ad libitum-fed animals of the same age

while urinary creatinine, osmolarity of urine, and transport of PAH by kidney slices, as well as the activity of renal sodium-potassium-activated adenosine triphosphatase would be higher.

CHAPTER II

REVIEW OF LITERATURE

I. FEED RESTRICTION AND ITS EFFECTS ON GROWTH, HEALTH AND LONGEVITY

As early as 1935, McCay and coworkers (1) demonstrated an increase in life span when rats were fed from weaning a diet adequate except for calories. In these studies, extreme underfeeding which allowed for stairstep growth of only 10 g every 2 to 3 months was used. In 1941, McCay et al. (2) reported that, of various dietary and exercise regimes introduced in middle life, those which were most beneficial to length of life were the ones which kept rats underweight by restriction of calories.

Carlson and Hoelzel (3), in work with intermittently fasted rats reported that extension of life span was practically proportional to the level of fasting. Their animals were fasted 1 day in 2, 1 day in 3 or 1 day in 4. They suggested that the optimum level of fasting might be 1 day in 3 since this caused 20% increase in life span of males and 15% in that of females without causing severe growth depression. Even in rats fasted 1 day in 2, although body weights were about 25% below those of controls, femoral lengths were not adversely affected.

Lane and Dickie (4) studied effects of feed restriction on genetically obese mice and compared them to both ad libitum-fed genetically obese mice and to ad libitum-fed non-obese mice. The degree of feed restriction was sufficient to keep the genetically obese mice the same weight as the non-obese controls. Of the 3 groups, life span was greatest

for the restricted animals. None of the ad libitum-fed obese mice lived beyond 667 days while 81% of the restricted and 60% of the non-obese mice lived beyond 730 days. These results show that a lower body weight was accompanied by an increase in life span with the longest life span being attained by limiting the intake of animals whose genetic make-up would have caused them to overeat instinctively if allowed feed ad libitum.

Widdowson and various coworkers (5,6,7) investigated the idea that accelerated growth might be undesirable, their interest in this having arisen from the fact that children seem to be growing faster and maturing earlier than did children in former years. Comparisons were made between rats which had been suckled in litters of 3 and those which had been suckled in litters of 15 to 20. After weaning at the age of 3 weeks, all animals were fed a stock diet ad libitum. In studies of rate of development (5), it was found that some characteristics, such as tooth eruption and eye opening, were age- rather than weight-related while others, such as sexual development, were related more to size than to age. Skeletal development (6) was not accelerated to the same extent in all bones of the fast-growing animals. Mean life span (7) of the slow-growing females living beyond 1 year was significantly greater than that of their fast-growing counterparts (119 weeks compared to 103 weeks), but there was no significant difference in life spans of males with different growth rates. Incidences of tumors, kidney disease and of enlarged hearts were more common in fast-growing animals. Still, the authors summarized their results as showing accelerated growth to be beneficial since more slow- than fast-growing animals died during the first year of life. It should be remembered, however, that in this study

restriction occurred during the period between birth and weaning while in other studies previously cited restriction was not begun until after weaning.

Roeder and Chow (8) recently reviewed the effects of undernutrition at an even earlier stage--maternal undernutrition as it affects the offspring. They considered undernutrition both in the form of reduction of total dietary intake and protein deprivation caused by either decreased quantity or decreased quality of the protein.

According to these authors (8) the first obvious result of maternal undernutrition, particularly when continued throughout both gestation and lactation, was an extremely high mortality rate of the young during postnatal life. The surviving progeny of such undernourished mothers had a poor weight gain and feed efficiency suggesting inadequate digestion, absorption and/or utilization of nutrients, and reduced learning ability with seemingly permanent effects on brain function. Various characteristics of the offspring of underfed rats indicated retarded development, a phenomenon which has been considered to be related to a slowdown of the aging process. However, according to Roeder and Chow (8) there is no evidence to support the idea that perinatal undernutrition, even when followed by ad libitum feeding throughout postweaning life, can be related to increased longevity. In fact, a later report by Roeder (9) pointed out that the rate of aging may actually be accelerated as a result of maternal restriction during gestation and lactation. For example, by middle age (19 months) increased catheptic activities of hepatic and renal tissues, biochemical alterations which accompany senescence in rats, were observed in perinatally undernourished rats.

Also, an accelerated rate of development of albuminuria was found in perinatally undernourished animals.

From the preceding discussion, it readily becomes apparent that the time at which feed restriction is initiated is vitally important since undernutrition in the very early developmental stages has been shown to be quite detrimental to the organism while restriction later in life may well be beneficial to health and longevity. Various investigators, including Radhakrishnan (10), Nolen (11) and Ross (12), have studied the effects of varying levels of restriction begun at varying times after weaning and sometimes followed by periods of repletion.

The work of Radhakrishnan (10) involved protein depletion and repletion rather than total calorie restriction. Restriction was accomplished by feeding rats a protein-free diet for a 4-week period beginning either the fourth, the twelfth, the sixteenth or the twentieth week of life. One group of rats was subjected to 2 periods of depletion which occurred in weeks 4 to 8 and 12 to 16. (The author compared the 4 week period of protein deprivation in the rats to a period of slightly over 3 years in the human population.) Protein depletion begun either the fourth or the twelfth week of life resulted in small body size of 16-week-old male rats, and the double depletion caused a reduction in size, including both body weights and measurements, in both male and female rats. The latter treatment also resulted in impaired reproductive performance of females, as measured by ability to produce young and birth weights of progeny. Protein depletion at the later periods of life caused temporary growth retardation which was reversed by subsequent adequate feeding.

Nolen's (11) experiment involved total dietary restriction after weaning and included rats in 7 different dietary groups. These groups included ad libitum-fed controls, animals fed either 80% or 60% of the ad libitum level throughout life, animals fed ad libitum for 12 weeks and then restricted to either 80% or 60% of the ad libitum level and animals restricted to either 80% or 60% for 12 weeks after which they were fed ad libitum. All treatments except the last 2 produced a decreased body size and mortality in both males and females as compared to controls with the greatest reduction of both body size and mortality being found in the 60% groups. Rats restricted early in life and then fed ad libitum had mortality patterns similar to controls but had higher levels of liver and body fat and smaller vital organs than controls. Nolen (11) suggested that the regimen of 80% restriction throughout life might be the most desirable since it resulted in twice as many survivors at 2 years of age without significantly changing the characteristics of the animals.

Ross (12) used a more severe restriction having groups fed either ad libitum, 6 g, 8 g, or 10 g per day. Restriction was begun either at weaning or at 70, 300 or 365 days. Animals restricted in later life were fed at the levels of 6, 8 or 10 g per day while all animals restricted from weaning were fed only 6 g per day. One group of animals was switched from restricted to ad libitum feeding at 70 days of age. Intake of 6 g per day increased the life expectancy of young growing rats, but restricting mature rats to this extent produced a substantial decrease in length of life. Increasing food allotments to 8 g did increase life expectancy of the mature rats with a further increase of food to 10 g

giving even better results. Feed restriction imposed only from 21 to 70 days of age or begun only at 70 days had some beneficial effects though these were not as great as when restriction was begun early and continued throughout life. Ross suggested that the abrupt and persistent stress of severe caloric restriction and rapid weight loss might account for the poor results obtained when mature animals were restricted to an intake of 6 g per day and proposed that a more gradual schedule of restriction resulting in a lengthened period of weight loss might be advantageous.

Other studies by Ross (13,14) evaluated longevity of rats fed diets with 2 levels of protein and varying levels of carbohydrate and 1 level of carbohydrate with varying levels of protein. Fat, vitamin and mineral content of these diets were identical, and all diets were fed on both a restricted and a non-restricted basis. In general, restriction of diets was beneficial to all length of life parameters. Protein level of isocaloric diets had little effect. Any of the diets which restricted caloric intake enhanced life expectancy, but restriction of both protein and carbohydrate with a simultaneous restriction of caloric intake had the most pronounced effect, especially in later life.

Edington (15) compared the effects on longevity of exercise begun at 120, 300, 450 and 600 days of life with those of feed restriction at a level which caused sedentary animals to maintain body weight within the range of weights of the exercised rats. The exercise was found to be beneficial to the younger animals, those that started training before 400 days of age, but the exercised animals had a lower survival rate than their sedentary controls when exercise was initiated at a later time. This indicated the existence of a "Threshold Age" beyond which it is not

advantageous, at least in terms of survival rate, to initiate an exercise program. It also seemed to show that body weight alone did not determine longevity since exercised and feed-restricted animals had different survival patterns. In another study of the effects of feed restriction and/or exercise on growth and survival of rats, Yager et al. (16), too, found that exercise could be detrimental in some circumstances and beneficial in others, with age and body weight being critical factors.

Berg (17) and Berg and Simms (18) studied feed restriction in relation to various parameters. Levels of restriction were 33 and 46% below ad libitum levels beginning after weaning. Weight reductions were due primarily to decreased body fat, and general health and fertility were better in restricted than in unrestricted rats. Onset of disease was delayed and longevity was extended by the feed restriction (18). Incidences of cardiac, renal and vascular lesions and tumors were significantly lower in restricted than in unrestricted animals at 800 days of age. Observations on rats up to 1200 days (19) showed that feed restriction continued to be beneficial although with advancing age, incidence of disease increased in both restricted and unrestricted animals.

Barrows and Roeder (20,21) and Barrows, Roeder and Fanestil (22) studied the effects of 50% restriction of dietary intake on life span and activities of various enzymes of rat tissue. One of the few consistent findings was an elevated hepatic succinoxidase level with restricted dietary intake. Restriction of weanling animals caused an increase in life span, but initiation of restriction at 12 months caused a slight decrease in life span, a finding which agrees with that of Ross (12). According to Barrows and Roeder (21), it seemed unlikely that the

enzymatic changes they observed could be responsible for alterations in life span since some of the same enzymatic changes were produced in both groups.

Halai (23) found that AKR male mice restricted to an intake approximately 33% below the ad libitum level had longer mean life spans than controls (403 days compared to 336 days). Biochemical parameters of restricted mice, including liver catheptic activity and percent of acid soluble collagen of the skin, resembled values obtained for chronologically younger ad libitum-fed animals.

Finch (24) recently reviewed the literature concerning enzyme changes and gene function associated with aging in mammals. According to his tabulations, few enzymes of the liver and kidney (the only organs which have been studied extensively enough to allow valid comparisons) show greater than a 25% change during adult life, and 80 to 90% remain essentially unchanged throughout life. There is also a wide variety of evidence that gene function remains generally unimpaired during aging. However, changes in endocrine activities with age, changing extracellular environment, may cause changes in cell activities. Finch pointed out the need for distinguishing between consequences of disease and consequences of aging, a task which is often complicated by pathological changes which are known to be age related--as amyloidosis and glomerulonephritis in kidneys of aging rats. Histological examination of tissues can be helpful in making this differentiation.

II. KIDNEY FUNCTION IN AGING HUMAN SUBJECTS

Much of the interest in kidney changes with age has stemmed from early work by Davies and Shock (25) who studied kidney function in 70 male subjects ranging in age from 24-89 years. In this group of subjects, it was found that glomerular filtration rate (as measured by inulin clearance) of 80-90 year old men was only about half that of men in the 20-30 year age group, and renal plasma flow (measured by diadrast clearance) showed a similar relationship. In a later paper, Shock (26) reported the following comparisons between kidneys of a 75 year old man and those of a 35 year old: number of glomeruli in the kidney, 56%; glomerular filtration rate, 69%; and kidney plasma flow, 50%. Shock suggested that the decreased blood supply to the kidney might be an adaptation mechanism permitting an increased flow to the brain.

Andrew (27) listed 3 main types of gross changes found in senile human kidneys. These included atrophy, capsular thickening and adhesion, and surface irregularities, and were accompanied by glomerular, tubular, vascular and interstitial changes of a histological nature. Glomerular changes ranged from congestion to fibrous or hyaline transformation. Distention and atrophy were the major tubular changes. Vascular changes were particularly found in the small arteries, and interstitial changes were mainly sclerosis of connective tissue in the cortex. However, Andrew (27) cited several references questioning these generalizations and concluded by saying that human studies really leave us uncertain as to whether any of the changes occur consistently enough to be designated definitely as due to aging or whether so-called senile changes are always associated with general arteriosclerosis in some sort of cause and effect relationship.

Tauchi et al. (28) compared senile changes in kidneys of Caucasian and Japanese male autopsy cases from 20-90 years of age. Changes found were toward decreased weight, decreased cell number and increased arteriosclerotic changes, fibrosis and hyaline changes with age in both races, but changes occurred at different rates in the 2 groups. Contrary to results concerning arteriosclerosis of the coronary arteries, the sclerotic changes of the intrarenal arteries were more severe with advanced age in the Japanese than in the Caucasian kidneys. The authors hypothesized that nutritional factors might play an important role in the race differences which occur in the process of senile changes in the kidney.

Inoue and coworkers (29) looked at acid mucopolysaccharide levels in 20 human kidneys from cadavers ranging in age from 1-84 years. They found an increase in acid mucopolysaccharides during the first 5 decades and a decrease thereafter. Cortical acid mucopolysaccharide levels were much lower and did not appear to be age related. The data from the renal medulla suggested that connective tissue metabolism might undergo some type of adaptive process with advancing age.

Lapides and Zierdt (30) questioned the generalization that renal function of normal healthy people decreased with age since they had observed many elderly people with normal glomerular and tubular function. To investigate the problem, they compared renal function of subjects above 65 years of age with that of subjects below 65. Their tests included glomerular filtration rate, derived from an inverse relationship with serum creatinine levels, and tubular function rate, determined from a 15-minute phenol-sulfonphthalein dye (PSP) excretion test. Their results showed 31% of the over-65 patients demonstrated 100% of both

glomerular and tubular function. Moderate impairment of glomerular function occurred about equally in subjects of the 2 age groups, but extensive damage was about twice as common in the older group as in the younger. However, of the 5 subjects over 90 years of age, 3 had perfect glomerular and tubular function, and 4 had normal glomerular function. Lapides and Zierdt (30) summarized their results by saying that a higher incidence of normal renal function will be found in younger people than in older ones, but they objected to the idea that renal function deteriorates with senescence per se and independently of disease processes though decreased renal function is notably associated with many diseases whose incidence is known to increase with age. This actually agrees fairly well with the earlier work of Davies and Shock (25) which recognized a wide range of individual differences including some normal kidney function in the oldest groups of subjects but with a general trend toward a decline in function with age.

Papper (31) recently summarized the effects of age in reducing renal function and discussed the clinical implications of these changes. He discussed the decreased concentrating and diluting ability which occur with age and the slowed reaction of the older kidney in adjusting to increased acid or alkaline loads. He speculated that the aged kidney, like the clinically diseased kidney, probably has a reduced number of functioning nephrons rather than most or all of the nephrons being defective. A few of the clinical implications include the potential for excessive water loss and subsequent dehydration with reduced fluid intake or extrarenal loss, the necessity for close monitoring of effects of any therapy since renal adjustment to alkalosis and acidosis are rather slow,

and the need for careful attention to detail in any clinical situation where body fluids may be altered.

III. KIDNEY FUNCTION IN AGING RATS

In a study of kidneys from young (75-100 days), middle-aged (300 days) and senile (868-1170 days) rats, Andrew and Pruett (32) found senile kidneys contained a colloid-like material which they assumed to be precipitated plasma proteins. This material was found to occur anywhere from Bowman's capsule to the larger collecting tubules. Aberrent cells were found in various segments of the nephron, and arteries contained deposits of lymphocytes, plasma cells and calcium salts in the adventitia (outer coat of the artery). Clear areas, probably caused by deposits of fatty material, frequently occurred in the media of the arteries. The glomerular tufts of the senile animals were, on the average, considerably larger than those of younger animals. There was little fibrosis of glomeruli in even senile animals of the study. Neither was arteriosclerosis, often found in the senile human kidneys, observed to any great extent in the rats.

In a histochemical study of tissue from adolescent and senescent rats, Rosenquist and Bernick (33) found a thickening of the tubular and glomerular basal laminae with age. Changes in the basal laminae included a decrease in acid mucopolysaccharides and sialomucin and an increase in neutral mucosubstances and collagen/reticular fiber ratio. It was suggested that the observed changes might be a result of hypoxia which might in turn be traceable to a decrease in blood supply.

Lesions of chronic nephrosis and glomerulonephritis were included by Simms and Berg (34) in a list of the 5 chronic diseases of greatest importance in aging rats. They found that about 95% of their rats had moderate or severe renal lesions by 1100 days while at 205 days no animals had severe lesions and only about 2% had moderate ones.

Another study by Berg and Simms (18) compared the onset of lesions (including chronic glomerulonephritis) in rats fed ad libitum and others restricted to 33 or 46% below the ad libitum level of intake. At 747 days, 100% of the unrestricted male rats exhibited lesions of glomerulonephritis while at 809 days only 36% of the animals fed the 33% restricted diet had such lesions, and only 1 animal (13%) in the 46% restricted group had developed symptoms by 818 days. Incidence of glomerulonephritis was lower in females than in males in corresponding groups with all restricted females being completely free of lesions. The authors pointed out that maximum body size was certainly not consistent with delaying the onset of disease or with increasing longevity. Rather, in mild feed restriction, the latent period preceding the onset of lesions was extended, and longevity was increased. Therefore, there must be some metabolic factor related to feed intake which influences the susceptibility of aging tissues, such as the kidney, to disease.

Bras and Ross (35) studied kidney disease in the rats subjected to the dietary regimes described earlier by Ross (13,14) in which animals were restricted in protein, in carbohydrate or in calories to varying degrees. All the experimental groups showed a "remarkable" reduction in prevalence of progressive glomerulonephrosis. Caloric level seemed to be more important than did protein level in bringing about this reduction.

Adams and Barrows (36) and Beauchene, Fanestil and Barrows (37) reported a decreased ability of kidney slices from old rats to actively transport paraaminohippuric acid (PAH). The latter group also reported age associated decreases in the transport of alpha-amino isobutyric acid and in the activity of renal sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase).

In the Adams and Barrows study (36), it was found that with a concentration of 3.88 μg PAH/ml in the medium there was no age associated difference in the accumulation of PAH by kidney slices. However, in a medium containing 200 μg PAH/ml, there were marked agewise decrements when the maximal active PAH accumulation by the tissue was estimated after 60 minutes incubation. These decrements exceeded the decreases in the concentration of DNA, meaning that the ratios of maximum PAH accumulation and minimum work required per unit of DNA were reduced with age. These data might be interpreted to mean that age differences are more pronounced when there are increased demands on the kidney.

In the study by Beauchene and coworkers (37), attempts were made to determine whether the age associated changes of the kidney were specific for PAH. In order to do this, they measured the accumulation of alpha-amino isobutyric acid by kidney slices and the activity of Na-K-ATPase in kidney homogenate, in addition to PAH accumulation. Contrary to the findings of Adams and Barrows (36), the age differences in PAH uptake were apparent even during the first 15 minutes of incubation, and there was a high correlation between activity of Na-K-ATPase and PAH accumulation by kidney slices of individual animals. Alpha-amino isobutyric acid accumulation, which involves a different transport mechanism than does PAH

uptake, showed a significant decrease with age and was significantly correlated with PAH uptake in individual animals.

The hypothesized loss of nephrons with age in human subjects (31), when compared with the findings of Adams and Barrows (36) which indicated that kidney function decreased more with age than did renal DNA content of rats, indicated that the effects of age on kidneys in the 2 species might not be identical. However, the techniques used for the rat studies were different from those employed for human investigations. Therefore, Gregory and Barrows (38) conducted an animal study using clearance techniques similar to those used in humans in an effort to get data which could be used to make valid comparisons. A lack of an age effect on glomerular filtration rate as determined by inulin clearance agreed well with the very slight decline in renal cell number based on DNA analysis and indicated a minimal loss of nephrons with age in rats. The PAH clearance values were in good agreement with values for in vitro PAH transport obtained by others (36,37). All this seemed to substantiate the idea that there really is some difference in the effect of age on kidney tissue of the 2 species although a decreased function with age has been found in both.

Zeman (39) observed the effects of maternal protein restriction on the structure of kidneys of newborn rats, and Hall and Zeman (40) studied kidney function in these animals. The kidneys of these animals were found to be immature with fewer identifiable glomeruli (39), lower glomerular filtration rate and depressed urine excretion during either water or osmotic diuresis (40). According to Allen and Zeman, increased postnatal nutrient intake had not completely reversed the retardation in

kidney development caused by prenatal protein deficiency by the time the rats were 90 days old (41), and it only partially compensated for the reduction in function (42). However, these studies are comparable to others employing restriction during gestation and/or lactation (5,6,7,8,9) which caused continuing detrimental effects and/or shortened life span and should not be confused with studies employing restriction at a later period of life and associated with an increased life span.

IV. AGE ASSOCIATED URINARY CHANGES IN RATS

The idea that there might be a decreased ability of tubule cells of older animals to perform osmotic work was suggested by a study conducted by Dicker and Nunn (43). These authors reported a decrease in the ability of older animals (350 days) as compared to controls (100 days) to concentrate urine after being deprived of water for 24 hours. The mean urinary excretion of older animals was 0.97 ml/100 g/24 hours compared to 0.62 ml/100 g/24 hours for the younger ones.

In other early work with kidney function in aging rats, Everitt (44) found that urine production remained relatively constant from youth to old age (750 days) but increased (in terms of output of urine per unit of metabolic body size) between 750 and 953 days. Consumption of feed and production of both urine and feces decreased progressively during the last 200 days of the life of the animal. Consumption of water per unit of metabolic body size decreased from youth to middle age, and then increased from old age to senility.

In a second paper, Everitt (45) reported urinary protein excretion approximately doubled from youth to middle age and again from middle

age to old age. Protein excretion of senile rats showed a 10-fold increase from youthful excretion. Everitt described massive protein excretion as an indication of renal disease, but the observed decrease in creatinine excretion was considered an age change. Excretion of uric acid and non-protein nitrogen were also considered to be age related and increased in middle age and again in the last 200 days before death. The changes in excretion of non-protein nitrogen, creatinine and uric acid associated with age were found not to be significantly different in high and low protein excretors.

Beauchene and coworkers (46) also reported an increase in proteinuria and an increased proportion of albumin in urinary proteins of old rats, changes thought to be due to the increased incidence with age of glomerulonephritis in the animals. The project, designed to study the uptake of labeled compounds by proteins of the liver and kidney in order to test the "Error Theory of Aging," showed a lack of effect of age on renal protein synthesis but an increase in synthesis of protein by the liver. The increased liver synthesis was considered to be secondary to the proteinuria found to occur in senescent rats rather than to changes in the structure of DNA molecules since there was a significant positive correlation between urinary albumin excretion and the incorporation of labeled precursors into liver protein and serum albumin.

Perry (47) had previously studied the urinary proteins excreted by the Wistar rat. Proteinuria was observed in all male rats by 8 weeks of age being within the range of 25-100 mg/100 ml of urine initially and showing an increase with age until rats 12-24 months of age excreted 1000-3000 mg protein/100 ml of urine. Such proteinuria was not found in

young female rats, leading the author to believe that the protein excreted by young males might be of testicular or prostatic origin. Separation of proteins by electrophoresis revealed a urinary protein having the same mobility as that found in the prostate and seemed to bear out the hypothesis. By 6-8 months of age, however, albuminuria was found in both males and females, and it increased with age. Simultaneous decreases in the serum albumin fraction and increases in the alpha-globulin fraction, changes consistent with nephrosis, were also observed.

Linkswiler et al. (48) also reported proteinuria in rats and agreed with the findings of Perry (47) that females and castrated males excreted less protein than normal males. Other factors found to influence protein excretion included level of casein in the diet and basal metabolic rate. Deficiencies of pantothenic acid, vitamin E and methionine were associated with decreased proteinuria while in vitamin B_6 deficiency, protein excretion was markedly elevated. Rats with liver tumors were also found to excrete large amounts of protein.

Berg (49) studied the relationships between renal lesions and changes in urinary proteins and serum proteins and cholesterol in rats from weaning until 640 days of age. By 240 and 370 days, lesions were occurring with increasing frequency exhibiting characteristics corresponding to those of nephrosis. Incidence and severity of lesions were greater at 640 days than at 370 days. At all ages, glomeruli were much more extensively involved than tubules. As proteinuria increased, electrophoretic patterns approached that of serum proteins. Serum albumin decreased slightly accompanied by a marked increase in serum

globulin with advancing age. Serum cholesterol values at 640 days were about twice those of young animals. There was no significant change in blood urea nitrogen with age. The findings of glomerular lesions, proteinuria and hypercholesterolemia are all consistent with findings in nephrosis. However, rats did not exhibit the edema and hypoalbuminemia which also help to characterize nephrosis in humans.

V. SODIUM-POTASSIUM-ACTIVATED ADENOSINE TRIPHOSPHATASE

Two papers by Katz and Epstein (50,51) attempted to clarify the role of Na-K-ATPase. The earlier paper (50) reviewed the evidence supporting the idea that Na-K-ATPase is involved in the active transport of electrolytes across membranes in tissues of many different types. Characteristics of the cell-membrane transport system of most animal cells include a requirement for ATP as an energy substrate, action upon monovalent cations and inhibition by digitalis glycosides, such as ouabain.

In 1962, Wheeler and Whittam (52) reported that properties of a kidney ATPase suggest that this enzyme is stimulated and inhibited in a way similar to active transport. Their evidence included such facts as the stimulation of the rate of ATP hydrolysis when sodium and potassium ions were added together to a rabbit-kidney cortex homogenate and removal of this stimulation of ATP hydrolysis with the addition of ouabain.

According to Katz and Epstein (50) most of the Na-K-ATPase activity of the kidney is contained in the homogenate fraction containing cell membranes and in the microsomes. These investigators found (51)

that Na-K-ATPase activity changed in an adaptive way when renal sodium resorption was chronically altered either up or down. Contralateral nephrectomy, high dietary protein intake and greatly reduced glomerular filtration rate caused by administration of an adrenocortical steroid all caused increased tubular reabsorption and increased Na-K-ATPase activity while adrenalectomy produced opposite effects on glomerular filtration and reabsorption of sodium and on Na-K-ATPase activity. These data appear to support the hypothesis that the Na-K-ATPase enzyme system is closely involved in the process of actively transporting sodium across the renal tubule.

Hendler, Torretti and Epstein (53) reported a higher level of Na-K-ATPase activity in the outer (red) medulla than in the cortex or in the white medulla of the kidney. Since the ascending limb of the loop of Henle, found in the red medulla of the kidney, handles a reabsorptive load per unit of tissue 8-16 times as high as that of the proximal tubule, the authors believed that the higher concentration of Na-K-ATPase, which is unaccompanied by increases in other membrane-bound enzymes, could be justified in terms of need. Another possible explanation is that sodium may actually be transported in at least 2 different ways by renal tubular cells, 1 of these ways being Na-K-ATPase dependent and the other Na-K-ATPase independent. If this were the case, these data suggest that the Na-K-ATPase-dependent pathway predominates in the ascending limb and distal tubules with the other mechanism predominating in the proximal tubule.

Whittembury (54) presented evidence for the case of 2 transport mechanisms, both of which seemed to be ATP-requiring and closely linked to respiration. He showed that ouabain selectively inhibited sodium for

potassium exchange but not the extrusion of sodium accompanied by chloride, but ethacrynic acid inhibited mainly the extrusion of sodium accompanied by chloride.

Chignell, Roddy and Titus (55) found that Na-K-ATPase levels in kidney homogenates from adrenalectomized rats were only about half the levels in homogenates from control animals. More recently, Hendler and coworkers (56) worked with influences of adrenalectomy and hormone replacement in rat kidney tissue. They were interested in seeing whether hormone effects were specific for Na-K-ATPase or whether they had a general effect on the plasma membrane. Their data showed a specific influence of glucocorticoids (methylprednisolone) in restoring Na-K-ATPase activity in adrenalectomized rats without altering levels of adenyl cyclase or 5'-nucleotidase (other enzymes of the plasma membrane). They suggested that the change was probably associated with an actual increased quantity of the Na-K-ATPase per unit of material.

VI. PARAAMINOHIPPURIC ACID TRANSPORT

Cross and Taggart (57) in 1950 reported a classical study of the mechanism for transporting PAH using renal cortical slices, and in 1959, Foulkes and Miller (58) proposed a model of this transport system. Their model consists of the following 4 steps: first, the diffusion of PAH from the medium into the extracellular space of the tissue; second, facilitated diffusion at the peritubular cell membrane; third, a build-up of a high tissue concentration within the cell; and fourth, a transfer of PAH across the luminal border of the cell into the tubular lumen. From the final step the PAH might then diffuse back into the medium.

According to a more recent article by Sheikh and Møller (59), with data on PAH transport by separated renal tubules (which eliminates complicating features such as the existence of extracellular space and the possibility of transport being slowed by a diffusion barrier between the external and internal parts of a slice) a short initial period of rapid transport was considered to be the active transport and was followed by a slowed uptake of PAH which may be due to intracellular compartmentation. These results are contradictory to the earlier model proposed by Foulkes and Miller.

Tanner and Isenberg (60) have discussed the meaning of in vivo PAH clearance. At low plasma levels of PAH, the PAH is removed almost completely from the plasma as it passes through the kidneys meaning that PAH clearance is approximately equal to renal plasma flow. However, at high plasma levels, the amount of PAH secreted per unit of time is maximal and is independent of the concentration in the plasma. The existence of such a maximal rate of tubular transport has been attributed to saturation of a limited number of cell transport sites for PAH. If this maximum rate is dependent on the number of secretory sites, it may be considered an index to the amount of functional secretory tissue in the kidney. The study by Tanner and Isenberg (60) indicates that there is, in fact, a limited number of proximal tubule secretory sites which may be related to tubule length, among other things, and which would seem to account for the existence of a tubular maximum for PAH.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. GENERAL PLAN

Animals for this study were male rats of Wistar origin obtained as weanlings from the National Research Laboratories in Creve Coeur, Missouri. Animal groups are shown in Table 1. Animals in groups AY and RY were sacrificed at 12 months of age and other animals were sacrificed at 23-25 months.

All animals were multiple housed with 5 animals per cage in 16" x 11" x 8" wire mesh cages. All animals were provided water ad libitum. Diets are described in the next section. Cages were examined daily, and any dead animals, removed and autopsied. Rats were weighed every 2 weeks until they were 4 months of age and monthly thereafter. Feed intakes for each cage of 5 rats were measured every 2 months throughout the 2 year study.

II. DIETARY TREATMENTS

Dietary treatments are shown in Table 1. Groups AH and RH were fed a diet of modified human foods (MHD). The composition of this diet is shown in Table 2. The remaining groups were fed Wayne Lab Blox (LB), a commercial laboratory feed. All Lab Blox were ground to allow for easy removal of feed from cages of restricted animals. The nutritional values of these diets are compared to recommended nutrient levels for rats in Tables 3 and 4.

TABLE 1

ANIMAL GROUPS AND DIETARY TREATMENTS

Group	n	Diet	Dietary Treatment Period	
			1-12 mo.	12-24 mo.
A	25	Lab Blox[1]	ad libitum	ad libitum
R	25	Lab Blox	restricted[2]	restricted
AR	30	Lab Blox	ad libitum	restricted
RA	30	Lab Blox	restricted	ad libitum
AY[3]	15	Lab Blox	ad libitum	--
RY[3]	15	Lab Blox	restricted	--
AH	25	Modified Human Diet[4]	ad libitum	ad libitum
RH	25	Modified Human Diet[4]	restricted	restricted

[1]Wayne Lab Blox--ground to facilitate use.

[2]Feed restriction was accomplished by making feed available 15 out of each 48 hours after an initial adaptation period.

[3]These animals were sacrificed at 1 year of age to serve as young controls.

[4]The composition of this diet is given in Table 2.

TABLE 2

COMPOSITION OF MODIFIED HUMAN DIET

Dietary Component	Percent of Diet
Lean ground beef[1] (cooked and dried)	5
Lean ground pork[1] (cooked and dried)	5
Nonfat dry milk[2]	17
Dehydrated sweet potatoes[3]	1
Dehydrated white potatoes[4]	21
Mixed greens[5] (dried)	1
Enriched white bread[6] (dried)	20
Cracked wheat bread[6] (dried)	5
Corn bread[7] (dried)	25

[1]East Tennessee Packing Company, Knoxville, Tennessee.

[2]Sugar Creek Foods, Knoxville, Tennessee.

[3]Fancy Yam Flakes, Instant Sweet Potatoes, NIFDA, Inc., Atlanta, Georgia.

[4]Mashed Potato Granules, Vitamin C added, NIFDA, Inc., Atlanta, Georgia.

[5]Grown locally.

[6]Merita Bread Company, Knoxville, Tennessee.

[7]Made with Three Rivers Self-Rising Cornmeal Mix, Enriched/ Bolted White, The White Lily Food Company, Knoxville, Tennessee.

TABLE 3

NUTRIENT COMPOSITION OF DIETS

Nutrient	Per 100 g of Diet		
	Recommended for Growth[1]	Lab Blox[2]	Modified Human Diet[3]
Calories	444.00	275.00	411.00
Protein,[4] g	13.30	24.50	21.50[5]
Carbohydrate, g	--	--	61.30
Fat, g	5.50	4.20	6.90[5]
Calcium, mg	560.00	1200.00	356.00[5]
Iron, mg	3.90	34.00	2.80
Vitamin A, IU	200.00	1500.00	1374.00
Thiamin, mg	0.14	1.40	0.36
Riboflavin, mg	0.28	0.65	0.58
Niacin, mg	1.67	6.00	4.20

[1]NRC Committee on Animal Nutrition (61).

[2]Laboratory animal feeding the Wayne way. Allied Mills, Inc.

[3]Based on values in U.S.D.A. Handbook No. 8 (62).

[4]Essential amino acid composition given in Table 4.

[5]Values obtained from actual analysis of diet samples.

TABLE 4

ESSENTIAL AMINO ACID COMPOSITION OF DIETS

Amino Acid	g Per 100 g of Diet		
	Recommended for Growth[1]	Lab Blox[2]	Modified Human Diet[3]
Arginine	0.7	1.4	1.4
Histidine	0.3	0.6	0.6
Isoleucine	0.6	1.4	1.2
Leucine	0.8	1.8	2.1
Lysine	1.0	1.6	1.5
Methionine	0.7	0.5	0.5
Phenylalanine	0.9	1.2	1.1
Threonine	0.6	1.0	1.0
Tryptophan	0.2	0.3	0.3
Valine	0.7	1.4	1.3

[1]NRC Committee on Animal Nutrition (61).

[2]Laboratory animal feeding the Wayne way. Allied Mills, Inc.

[3]Calculations based on values given in Home Economics Research Report No. 4 (63).

The diet of modified human foods was prepared in the following manner:

Ground meat was cooked in a frying pan until brown with fat being continually dipped from it. The meat then was transferred to shallow pans lined with absorbent paper and dried in an oven at approximately 95° for 4 to 6 hours or until it appeared dry when crumbled. It was then cooled and stored at -10° until needed.

Raw greens were dried at 45° in a convection oven overnight (approximately 15 hours). They were then ground with an institutional coffee grinder.

Slices of white and cracked wheat bread were placed on flat pans and dried in an oven at 95° for 4 hours. Bread was then crumbled and ground with a coffee grinder.

Corn bread was made by the following recipe:

2600 g cornmeal mix	6 large eggs
400 g nonfat dry milk	1 1/2 c Wesson oil
1 g vitamin D oil (40,000 IU/g)	8 1/2 c water

Eggs were beaten with oil. Then all ingredients were mixed together using an institutional electric mixer, poured into pans, and baked at 188° for 1 hour. Bread was then crumbled, spread in shallow pans, dried at 95° for 4 hours and ground with the coffee grinder.

Other ingredients of the diet were purchased in the form to be used. All dietary ingredients were mixed, ground and frozen at -10° until needed.

Feed restriction was accomplished by having feed available to the animals for only a limited period of time. At the final level of

restriction, feed was made available for only 15 out of each 48 hours (5:00 PM to 8:00 AM on alternate days). However, weanling rats had previously been observed by the author to be unable to survive and grow on this type schedule. Therefore, a gradual adaptation process was used to achieve this pattern of restriction for feeding the adult animals. Rats were fed for 24 out of 48 hours beginning at 1 month of age. At 8-day intervals, feeding time was cut back to 21, 18 and finally 15 out of 48 hours until by 2 months of age, animals were eating according to the permanent schedule. When rats of group AR were switched to restricted feeding at the age of 12 months, the adult animals were adapted to feed restriction in a similar manner.

III. URINE COLLECTION

At 3, 9, 12, 15 and 21 months of age, 12 randomly selected animals from each of groups A, R, AR, RA, AH and RH were placed in individual metabolism cages for the collection of 4-day (96 hour) urine specimens to be analyzed for protein and creatinine content. Collections were made from animals of groups AY and RY only at 12 months of age, immediately before sacrifice.

Bottles for urine collection were prepared by pipetting 0.2 ml of thymol in propanol (10% w/v) into each bottle (64) and adding 2 drops of light weight mineral oil by dropper to each bottle (65). Each collection bottle was then placed in a clean 600 ml beaker and positioned below the outlet of the metabolism cage so that urine went into the bottle and feces into the beaker.

Collection bottles were replaced, and funnels were rinsed daily. Waxed paper was placed below each metabolism cage to catch any excretions during the washing period. Any urine collected in this manner was then added to the total sample.

Urines were centrifuged daily at 800 x g in an International Model SBB centrifuge for 10 minutes to remove feed which might have fallen into the collection bottles. Centrifuged urines were composited for each rat and kept refrigerated until the end of the 4-day collection period. Total volumes were measured, and urines were frozen at -10° until needed for analysis.

Osmolarity was determined on fresh urines from the last collection before sacrifice.

IV. URINE DETERMINATIONS

Osmolarity

Samples of 2 ml each were taken from composite collections of urine. Osmolarity (expressed as milliosmoles per liter) was determined using a Precision Osmometer. The principle of the instrument involves measurement of the depression in freezing point depending on the concentration of molecules and ions in solution.

Urinary Proteins

Urines were tested qualitatively for protein by adding a few drops of 3% (w/v) sulfosalicylic acid to an equal amount of urine (66). Results of this test determined the sample size to use in the quantitative test. If the sulfosalicylic acid test gave a slight turbidity, 0.8 ml of urine was used. If a light precipitate was formed, 0.6 ml of urine was used, and if a heavy precipitate, 0.4 ml of urine.

The quantitative test used was that of Saifer and Gerstenfeld (67) except that biuret reagent (68) was used for color development. The biuret test depends on the formation of complex ions between copper and proteins in an alkaline solution to give a violet color which can be measured spectrophotometrically.

Reagents.

1. Biuret Reagent: 3 g $CuSO_4 \cdot 5H_2O$, 9 g $NaKC_4H_4O_6 \cdot 4H_2O$, 8 g NaOH and 5 g KI were dissolved and diluted to 1 liter with water.

2. Bovine Serum Albumin Standard: 1 g bovine serum albumin (BSA) was dissolved in and diluted to 100 ml with 5% (w/v) NaCl. Working standards were prepared daily using 0-0.8 ml of the stock standard.

3. Perchloric Acid (PCA) Solution, 0.14% (w/v): 0.4 ml of 70% PCA was diluted to 200 ml with acetone immediately before use.

Procedure. Standards and duplicate urine samples (sample size determined as described above) were diluted to 1 ml using 5% NaCl. To this was added 4 ml of the PCA-acetone mixture. Samples were then mixed using a Vortex Genie Mixer, incubated in a 50° water bath for 20 minutes, and centrifuged at 800 x g for 12 minutes in an International Model SBB centrifuge. The supernatant fluids were discarded, 2 ml 5% trichloroacetic acid (TCA) was added, and samples were again mixed, centrifuged and carefully drained. Protein pellets were dissolved in 1 ml of 0.1 N NaOH and incubated in a 37° water bath for 15 minutes. Four ml biuret reagent was added to each sample. Samples were mixed, incubated for 15 minutes at 37°, and absorbances were determined at 550 nm using a Beckman B spectrophotometer. Protein excretion per 24 hours was calculated as follows:

$$\text{mg protein/24 hr} = \frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times \frac{1}{\text{sample size}} \times \text{24 hr urine vol}$$

Urinary Creatinine

This determination described by Henry (69) is based on the measurement spectrophotometrically of the red pigment, creatinine picrate, formed when picric acid and NaOH are added to a solution containing creatinine.

Reagents.

1. Oxalic Acid: a saturated solution was prepared with water.

2. Lloyd's Reagent: a 10% (w/v) suspension was prepared with water.

3. Creatinine Standard: 500 mg creatinine zinc chloride was dissolved in 0.1 N HCl and diluted to 500 ml with the HCl. This stock solution was diluted daily in a ratio of 1.5:100 with water. Working standards were prepared using 0-4.5 ml of the diluted standard.

Procedure. One ml of each urine sample was pipetted into a 15 ml conical centrifuge tube and diluted with 7 ml of water, 1 ml of 10% (w/v) sodium tungstate and 1 ml of 2/3 N H_2SO_4. Samples were then mixed and centrifuged at 800 x g for 10 minutes in an International Model SBB centrifuge.

A final dilution of 1:100 was obtained by pipetting 2.5 ml of the deproteinated urine into a 25 ml volumetric flask and diluting to volume with water. Standards and duplicate 3 ml samples were diluted to 5 ml with water, and to each was added 0.5 ml saturated aqueous oxalic acid and 1 ml 10% aqueous suspension of Lloyd's Reagent. Samples were mixed intermittently for 10 minutes using a Vortex Genie Mixer, centrifuged at 800 x g for 12 minutes and drained. Then 3 ml water, 1 ml 1% picric acid and 1 ml 0.75 N NaOH were added to each tube, and the mixing and centrifuging steps, repeated. The absorbances of the

supernatant fluids were measured at 520 nm using a Beckman B spectrophotometer. Urinary creatinine excretion per 24 hours was calculated using the following formula:

$$\text{mg creatinine/24 hr} = \frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times 0.033 \times \text{24 hr urine vol}$$

V. PREPARATION OF TISSUE

All animals were allowed to eat ad libitum the night before sacrifice and were weighed shortly before sacrifice. Ad libitum-fed and restricted rats were killed alternately, and order was rotated daily to minimize bias resulting from holding time of tissue before analysis.

Rats were stunned by a blow on the head and then decapitated. A drop of blood from each rat was placed on an AZOSTIX[1] strip and read immediately for blood urea nitrogen. The rest of the blood was drained from rats into test tubes and held for about an hour before centrifuging to separate serum for the creatinine determination. Kidneys were removed and placed on chilled watch glasses where they were kept on ice until time for slicing. Rats were further examined for any abnormalities.

The clotted blood was loosened from the tube and centrifuged at 550 x *g* for 15 minutes in an International Model SBB centrifuge. The serum was removed with a dropper and put into a clean tube. The blood clot was removed with a small metal spatula and discarded. The blood was again centrifuged, and the additional serum, collected.

Kidneys were decapsulated and weighed to the nearest 0.01 *g* on a Mettler top-loading balance. A Stadie-Riggs microtome was then used to

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[1]AZOSTIX, Ames Company, Division Miles Laboratory, Inc., Elkhart, Indiana.

slice the left kidney for the PAH determination. The remaining portion of the left kidney was preserved in 3.7% formaldehyde solution and saved for slides. The right kidney was homogenized for the ATPase determination.

VI. KIDNEY DETERMINATIONS

Paraaminohippuric Acid Transport

The uptake by kidney slices of PAH from a suspending medium is an in vitro measure of the ability of the kidney to perform osmotic work. PAH released from a homogenate of the kidney slices was determined by a diazotization reaction followed by the addition of a reagent which reacts to yield a pink color which can be measured spectrophotometrically (36).

Reagents.

1. Medium, pH 7.3: 5.06 g NaCl, 2.98 g KCl, 1.36 g sodium acetate ($C_2H_3NaO_2 \cdot 3H_2O$), 1.98 g sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) and 200 mg PAH were dissolved in approximately 800 ml water, and the pH, adjusted to 7.3 with HCl. The solution was then diluted to 900 ml with water. A calcium chloride solution containing 0.11 g calcium chloride ($CaCl_2 \cdot 2H_2O$) diluted to 100 ml with water was mixed with the rest of the medium in a ratio of 1:9 immediately before use.

2. Sodium nitrite ($NaNO_2$): a 0.2% solution was prepared with water daily.

3. Ammonium sulphamate: a 0.5% solution was prepared with water.

4. N-(1-naphthyl)-ethylene diamine dihydrochloride (EDA): a 0.2% solution was made with water.

5. Paraaminohippuric acid (PAH) standard: 100 mg PAH was dissolved in 100 ml 0.1 N HCl. Ten ml of this standard was diluted to 100 ml with

0.1 N HCl, and this was diluted again to give a final working standard of 10 μg/ml. A series of standards were prepared daily by diluting 0-2.5 ml of the working standard to 6 ml with 0.1 N HCl.

Procedure. The buffer medium was mixed as described above, and 10 ml of medium was pipetted into each of a series of 25 ml Erlenmeyer flasks which were chilled in ice until incubation of the kidney slices. Kidneys were removed, weighed and sliced (5 mm thickness) with a Stadie-Riggs microtome. The outer (capsular) slices from each side of the kidney were discarded, and 2 inner slices from each side were weighed to the nearest 0.2 mg using a Roller-Smith balance and then dropped immediately into flasks containing medium.

Slices were incubated for 1 hour at 37° in an Eberbach water-bath shaker moving at the rate of 120 cycles per minute with oxygen flushed through them. Flasks were then removed from the shaker, and slices were lifted from the flasks with forceps. Excess medium was drained from slices against flask necks. Each slice was washed by dipping into physiological saline (0.9%), and again excess liquid was removed against the side of the container. Each slice was then dropped into a graduated centrifuge tube to which about 1 ml of water was added from a wash bottle.

Each slice was homogenized in 10% TCA using a tissue grinder with a Teflon pestle. Homogenates were diluted to a final volume of 7 ml in the graduated centrifuge tubes with 10% TCA. Samples were mixed with a Vortex Genie Mixer and then centrifuged at 800 x g for 10 minutes in an International Model SBB centrifuge.

Duplicate 2 ml samples of supernatant fluids from homogenates were pipetted into spectrophotometer tubes. To each sample was added 0.5 ml of 2 N HCl and enough water to bring the total volume in the tube to 4.5 ml. Tubes were topped with marbles, and samples were hydrolyzed for 30 minutes in a boiling water bath after which they were cooled to room temperature.

To each standard and sample was added, in order, 0.5 ml sodium nitrite, 0.5 ml ammonium sulphamate and 0.5 ml EDA. Samples were mixed after addition of each reagent and finally were diluted to a total volume of 7.5 ml with 0.1 N HCl. Samples were allowed to stand for 10 minutes before the absorbances were measured at 540 nm with a Beckman B spectrophotometer. PAH in the tissue was calculated as follows:

$$\mu g \text{ PAH/mg tissue} = \left(\frac{\frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times 3.5}{\text{mg tissue}} \right) - 200 \text{ ;}$$

$$\mu g \text{ PAH/mg protein} = \frac{\mu g \text{ PAH/mg tissue}}{\text{mg protein/mg tissue}} \text{ ;}$$

Total PAH/100 g body wt =

$$\frac{10 \text{ (}\mu g \text{ PAH/mg protein) (rt kidney wt + lt kidney wt, g) (percent protein)}}{\text{body wt, g}}$$

Tissue Protein

The method for the determination of tissue proteins was a modification of the method of Shibko et al. (70) and the biuret reagent (68) was used for color development. The principle for determination of protein using this reagent was described in Section IV of this chapter.

Reagents.

1. Biuret Reagent: described in Section IV, p. 34.

2. Bovine Serum Albumin Standard: 1 g bovine serum albumin (BSA) was dissolved in and diluted to 100 ml with 0.4 N NaOH. Working standards were prepared daily by diluting 0-0.8 ml of the stock standard to 1 ml with 0.4 N NaOH.

3. Perchloric Acid Solution (PCA), 0.6% (w/v): 5 ml of 70% PCA was diluted to 1 liter with 95% ethanol.

Procedure. The precipitates obtained after centrifuging the TCA-tissue homogenates of the PAH determination were drained. The precipitates were washed with 3 ml of 0.6% PCA solution and again with 3 ml of ether. Each time, the samples were mixed with a Vortex Genie Mixer, centrifuged at 800 x g for 5 minutes in an International Model SBB centrifuge, and the supernatant fluids discarded. After the final centrifugation, the protein pellet was dissolved in 3 ml of 0.4 N NaOH and left to stand overnight. To 1 ml of each of these protein solutions and each standard was added 4 ml of biuret reagent. The samples were mixed, incubated for 30 minutes in a 45° water bath, allowed to cool to room temperature and absorbances determined at 550 nm using a Beckman B spectrophotometer. Protein was calculated as follows:

$$\text{Percent protein} = \frac{\frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times 3}{\text{wt of slice}} \times 100$$

Sodium-Potassium-Activated Adenosine Triphosphatase

Microsomal suspensions prepared from kidney homogenates were incubated with ATP according to the procedure described by Hendler and coworkers (56). ATPase activity was determined by analysis for inorganic phosphate liberated. The difference between inorganic phosphate liberated in the presence and absence of potassium was considered to be the

Na-K-activated portion of the ATPase. Correction was made for spontaneous, nonenzymatic breakdown of ATP by measuring inorganic phosphate liberated under the same experimental conditions but without the presence of the enzyme.

Reagents.

1. Homogenizing Solution: 0.25 M sucrose, 6 mM disodium (ethylenedinitrilo) tetraacetate (EDTA), 20 mM imidazole and 2.4 mM sodium deoxycholate (added immediately before use). Forty-five g sucrose, 2.23 g EDTA and 1.36 g imidazole were dissolved in water. The pH of the solution was adjusted to 6.8, and water was added to give a final volume of 900 ml. This solution was frozen in polyethylene bottles and stored until needed. One g of sodium deoxycholate was dissolved in water and diluted to a final volume of 100 ml. The 2 solutions were mixed in a ratio of 9:1 immediately before use.

2. Incubation Medium: 100 mM NaCl, 20 mM KCl, 10 mM imidazole, 6 mM $MgCl_2$ and 6 mM disodium ATP. One aqueous solution was made using 5.85 g NaCl, 1.49 g KCl and 0.68 g imidazole. A second solution was made using the same components except omitting the KCl. The pH of each solution was adjusted to 7.8, and water was added to give each solution a final volume of 800 ml. Solutions were frozen in polyethylene bottles and stored until needed. Equal parts of $MgCl_2$ (1.22 g $MgCl_2 \cdot 6\ H_2O$ diluted to 100 ml with water) and disodium ATP (prepared daily using 0.825 g ATP diluted to 25 ml with water) were mixed and pipetted directly into flasks to start the reaction.

Procedure. A 5% (w/v) tissue homogenate was prepared with the homogenizing solution using a tissue grinder equipped with a Teflon pestle.

The homogenate was then filtered through a double layer of gauze and centrifuged for 30 minutes at 10,800 x g in a refrigerated Lourdes Beta-Fuge Model A centrifuge using a 9 RA Rotor. The supernatant fluid was decanted and recentrifuged for 40 minutes at 105,000 x g in a refrigerated Beckman Model L5-50 Ultracentrifuge using a general purpose Type Ti Fixed-Angle Rotor. Microsomes were resuspended in 1.5 ml of the homogenizing solution without deoxycholate and frozen overnight at -10° before analysis was completed.

Microsomal samples of 0.2 and 0.4 ml were pipetted into 8 ml of incubation medium (either complete or without KCl) in 25 ml Erlenmeyer flasks. The mixture was prewarmed for 5 minutes in a 37° Eberbach water-bath shaker moving at 120 cycles/minute. Then 2 ml of the $MgCl_2$-ATP mixture was added to each flask to start the reaction which was carried out for 15 minutes in the 37° water bath. The reaction was terminated by removing 8 ml of the mixture from each flask and immediately adding 1.6 ml of ice cold 35% (w/v) TCA to each sample. Samples were mixed with a Vortex Genie Mixer and centrifuged at 800 x g for 5 minutes in an International Model SBB centrifuge. The supernatant fluids were analyzed for inorganic phosphate by the method of Fiske and SubbaRow (71). Microsomal protein was determined using the method of Lowery et al. (72).

Inorganic Phosphate

Ammonium molybdate converts inorganic phosphate to phosphomolybdate which is then reduced with an amino-naphthol-sulfonic acid reagent forming a blue color which can be measured spectrophotometrically (71).

1. Molybdate Reagent: 12.5 g of ammonium molybdate was dissolved in 100 ml water. To this solution was added 150 ml of 10 N sulfuric acid. The solution was then diluted to 500 ml with water and stored in a polyethylene container.

2. Amino-Naphthol-Sulfonic Acid (ANSA) Reagent: 0.2 g of 1-amino-2-naphthol-4-sulfonic acid was dissolved in 78 ml of 15% (w/v) sodium bisulfite solution. To this was added 2 ml of 20% (w/v) sodium sulfite solution, and the solution was filtered.

3. Phosphate Standard: 1.098 g of potassium dihydrogen phosphate was dissolved in 2.5 ml of 10 N sulfuric acid and diluted to 250 ml with water. A working standard was made from this by diluting 3 ml of the stock standard to 100 ml to give a concentration of 3 mg/100 ml. A series of standards were prepared daily by diluting 0-0.8 ml of the working standard to 2.5 ml with water.

Procedure. One ml of supernatant fluid from the flasks was diluted to 10 ml with water. From each of these diluted samples, 2.5 ml duplicate samples were pipetted. Color development was achieved by adding 0.5 ml of molybdate reagent and 0.2 ml ANSA to each sample and standard, mixing after each step. Finally, samples were diluted with 3 ml of water and allowed to stand for 5 minutes before measuring their absorbances at 660 nm with a Beckman B spectrophotometer. Phosphate was calculated as follows:

$$\text{mg phosphate/mg protein} = \frac{\frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times 0.48}{\text{mg protein from flask}}$$

Microsomal Protein

Microsomal protein was determined using the method of Lowery et al. (72). This is a very sensitive method which utilizes the reaction of phenol

reagent with peptide bonds, tyrosine and tryptophan of protein to give a deep blue color which can be measured spectrophotometrically.

Reagents.

1. Sodium Carbonate Reagent: a 2% (w/v) solution of Na_2CO_3 was prepared using 0.1 N NaOH. Equal parts of 0.5% (w/v) $CuSO_4$ and 1% (w/v) NaK tartrate were combined. The second mixture was added to the Na_2CO_3 solution in the ratio of 1:100.

2. Phenol Reagent: a 1 N solution was prepared by mixing equal parts water and 2 N phenol reagent.

3. Bovine Serum Albumin: a stock solution of this standard was prepared by dissolving 500 mg of bovine serum albumin in 0.2 N NaOH and diluting to 100 ml. Working standards containing 0-0.4 mg/ml were prepared by diluting the stock standard with 0.2 N NaOH.

Procedure. Microsomal protein pellets were dissolved in 2 or 4 ml of 0.2 N NaOH depending on the size of the sample incubated. To 0.8 ml of samples and working standards was added 4 ml of the sodium carbonate reagent. Samples were mixed and allowed to stand for at least 10 minutes. Then 0.4 ml of phenol reagent was added to each as samples were mixed with a Vortex Genie Mixer. After 30 minutes, the absorbances of the samples were measured at 700 nm using a Beckman B spectrophotometer. Standard curves were plotted and concentrations of samples were read from the curves.

VII. BLOOD DETERMINATIONS

Serum Creatinine

The principle for creatinine determination is described on page 35. Reagents used were the same as those for determination of urinary creatinine, and the procedure used was similar (69).

Duplicate 2 ml samples of serum were pipetted into conical centrifuge tubes. Three ml of water, 1 ml of 10% sodium tungstate and 2 ml of 2/3 N H_2SO_4 were added to each tube. The contents of the tubes were mixed and then centrifuged at 715 x g for 10 minutes.

Standards were prepared using 0-0.8 ml of the creatinine zinc chloride standard described on page 35 containing 15 µg creatinine/ml. Standards and 3 ml samples of each protein-free centrifugate obtained in the above manner were diluted to 5 ml with water. Next, 0.5 ml of saturated aqueous oxalic acid and 1 ml of 10% aqueous suspension of Lloyd's Reagent were added to each sample. Samples were mixed intermittently for 10 minutes using a Vortex Genie Mixer, centrifuged for 12 minutes at 715 x g in an International Model SBB centrifuge, and drained. Three ml of water, 1 ml of 1% picric acid and 1 ml of 0.75 N NaOH were added to each tube, and the mixing and centrifuging, repeated. The absorbances of the supernatant fluids were measured at 520 nm using a Beckman B spectrophotometer. Serum creatinine levels were calculated using the following formula:

$$\mu g \text{ creatinine/ml serum} = \frac{\text{Conc of std}}{\text{A of std}} \times \text{A of sample} \times 1.33$$

Blood Urea Nitrogen

Blood urea nitrogen (BUN) was determined by use of AZOSTIX, plastic strips impregnated with urease and bromthymol blue. When blood is

applied to the impregnated area of the strip, urease catalyzes the hydrolysis of blood urea forming carbon dioxide and ammonium hydroxide and thus increasing the pH. The bromthymol blue changes color with this shift in alkalinity, and it is this color change that is used to determine the blood urea nitrogen level (73).

Procedure. A large drop of fresh whole blood was applied directly to the impregnated area of an AZOSTIX strip. After 60 seconds, the blood was immediately washed off using a wash bottle, and the color was compared to the chart provided. Interpolation was used when the color produced fell between 2 color blocks. Comparisons had to be done in less than 5 seconds after washing since color fades very rapidly. Results were read as mg BUN/100 ml blood.

VIII. GROWTH CURVES

Growth curves were calculated by computer from body weights taken at 16 points during the first year of an animal's life. The formula, developed by Brody (74), is given below:

$$W = A-Be^{-kt}$$

where W is animal weight in g at any given age;

A is mature weight measured in g;

B is an integration constant;

e is the base of natural logarithms;

k is rate of growth; and

t is age measured in weeks.

In this formula, k is the relative growth rate with respect to the growth yet to be made. The growth rate (k) declines at a constant percentage

rate, and the negative sign of k in the formula indicates this decline.

Values for A, B and k were calculated for individual animals, and means of these values were used for plotting growth curves for groups.

IX. HISTOPATHOLOGY

Sections of kidney tissue were preserved in a 3.7% formaldehyde solution, embedded in paraffin blocks and sliced for mounting. Slides were stained with hematoxylin and eosin stain.

Each kidney section was viewed under a microscope and assigned a rating of 1 to 5 depending on the number and severity of lesions. Descriptions of each rating on the scale are given below, and examples of sections receiving each rating are shown in Figures 1-5. All sections showed some age associated glomerular change in addition to the changes described below.

1 - few or no casts;

2 - some casts and minimal or focal areas of inflammation;

3 - many casts and slight inflammation;

4 - many casts and chronic diffuse inflammation;

5 - many casts, cysts, chronic active inflammation and interstitial fibrosis.

X. STATISTICAL METHODS

Analysis of data was done using standard statistical procedures (75). Analysis of variance was performed on variables to test for differences among treatments. Kramer's modification (76) of Duncan's

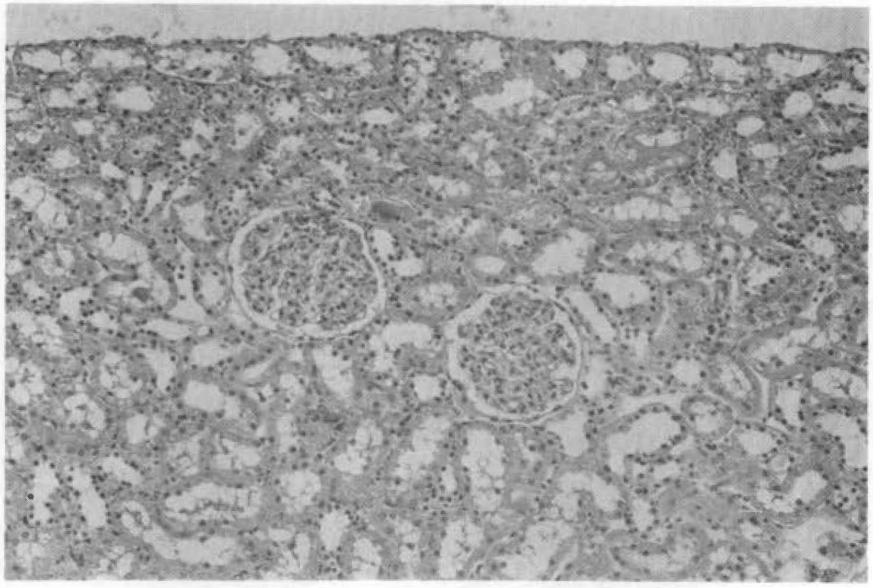


Figure 1. Kidney section showing glomerular thickening. Hematoxylin and eosin, x 180 (Scored 1).

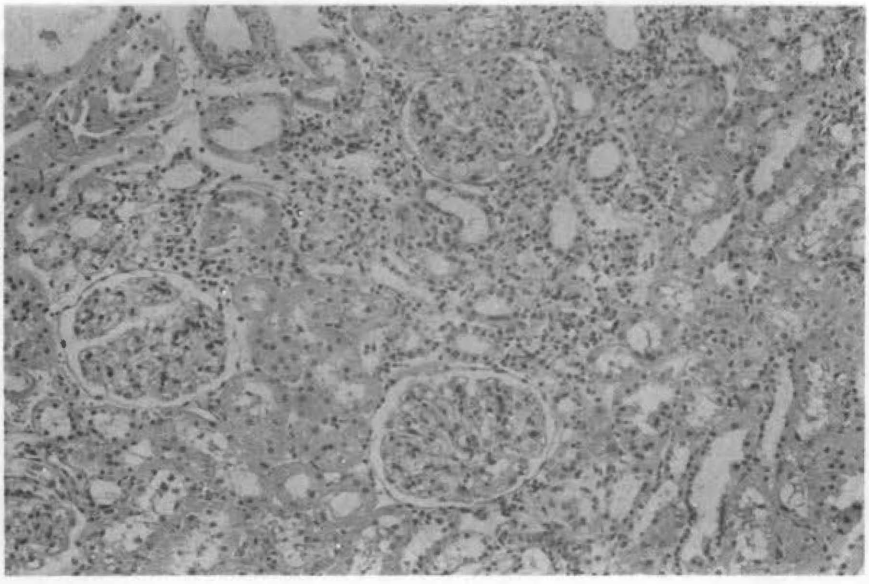
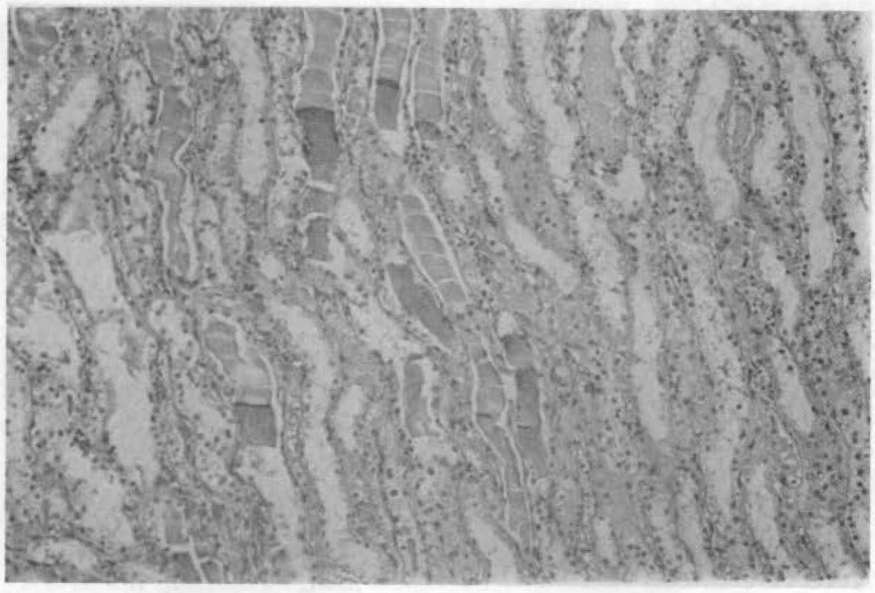


Figure 2. Kidney section showing minimal inflammation. Hematoxylin and eosin, x 180 (Scored 2).

A.



B.

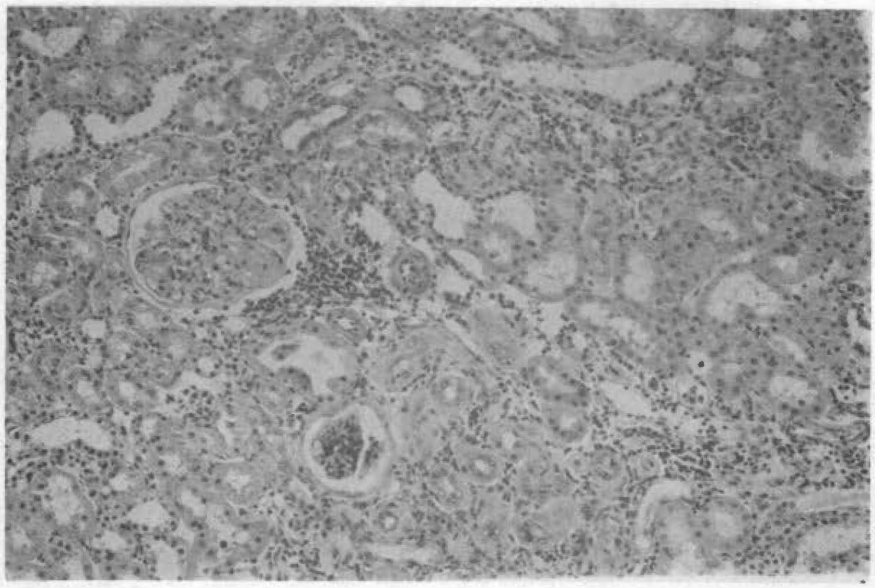


Figure 3. Kidney section showing many casts and slight inflammation. Hematoxylin and eosin, x 180 (Scored 3). A. Section showing many casts. B. Section showing slight inflammation.

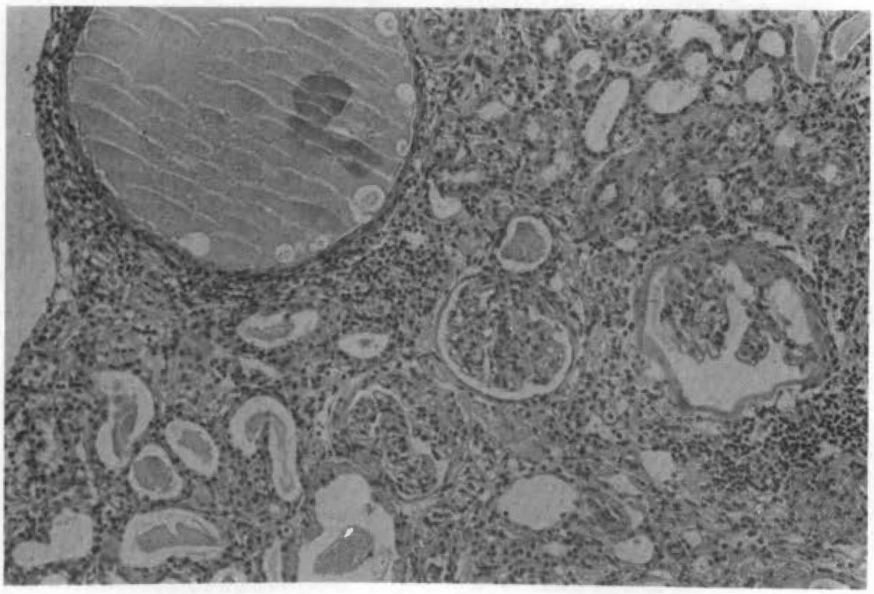


Figure 4. Kidney section showing many casts and chronic diffuse inflammation. Hematoxylin and eosin, x 180 (Scored 4).

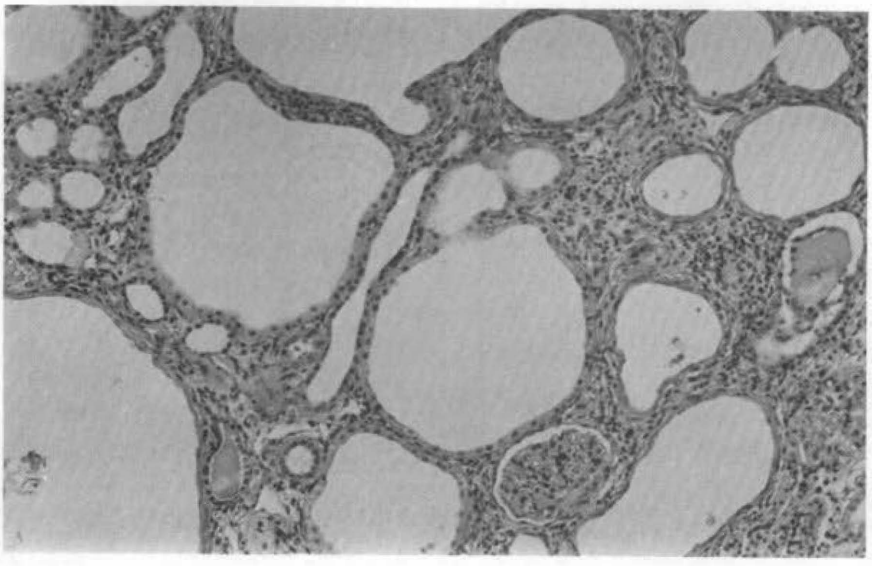


Figure 5. Kidney section showing many casts, cysts, chronic active inflammation and interstitial fibrosis. Hematoxylin and eosin, x 180 (Scored 5).

Multiple Range test was used to partition means found to be significantly different using the F-test with the analysis of variance. A coefficient of rank correlation was used to determine the relationship between urinary protein and pathology ratings. The Students t test was used for testing differences between means for some variables.

CHAPTER IV

RESULTS

Figure 6 shows mean daily feed intake per cage of 5 rats for groups fed Lab Blox (LB). Intakes are shown when animals were 10, 25, 51, 72 and 97 weeks old. For each period during the first year, intakes for the 3 ad libitum-fed groups (AY, A and AR) were averaged. Similarly, an average was found for the 3 groups of restricted animals (RY, R and RA). Comparison of feed intakes of ad libitum-fed and restricted rats during the first year refers to the averages. (This same practice will be continued in presenting other data collected at several periods unless otherwise indicated.) Groups AY and RY were sacrificed at the end of the first year; group AR was switched from ad libitum to restricted feeding; and group RA was switched from restricted to ad libitum feeding at this time. Therefore, results for the second year are reported for individual groups.

The mean feed consumption per cage of 5 restricted rats at 10 weeks of age was 55% (67 g compared with 120 g) of the level for ad libitum-fed animals eating LB, a difference which was statistically significant ($p < 0.05$). Feed consumption was still significantly lower ($p < 0.05$) for restricted than for ad libitum-fed animals at 97 weeks, but the percentage had increased to 65% of the ad libitum level (69 compared with 106 g). By 97 weeks, rats of group AR ate 80 g or 75% of the ad libitum level, an amount significantly different ($p < 0.05$) from intake of both group A and group R. Feed consumption of group RA at this

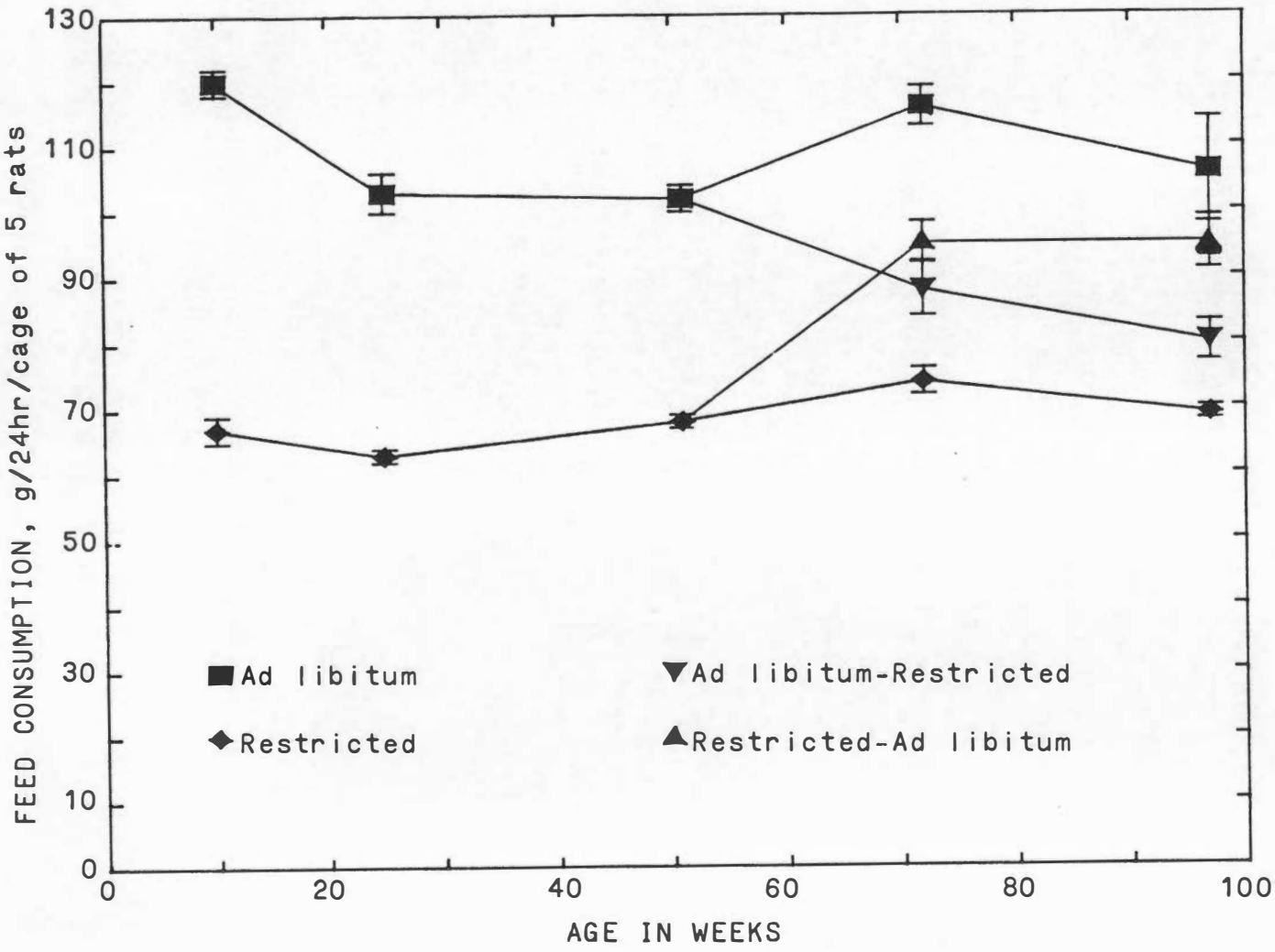


Figure 6. Influence of restriction on feed intake of animals eating Lab Blox. Points show means ± SEM.

time was 95 g or 90% of the ad libitum level, an amount significantly higher ($p < 0.05$) than that of group R but not different from group A.

Figure 7 shows that feed intakes of animals in group AH and RH were consistently lower than for animals in corresponding groups fed LB although the differences between restricted groups eating the 2 diets were not significant at 10 weeks of age. Restricted rats fed the modified human diet (MHD) ate 60% (59 compared to 97 g) of the level of the ad libitum-fed animals eating the same diet at 10 weeks of age and had increased to 68% (59 compared to 87 g) by 97 weeks, percentages slightly higher than the comparable figures for LB groups (see above).

The levels of restriction just described were not severe enough to cause a high mortality rate for any of the treatment groups. The percentages of animals from each group which died a natural death before 25 months of age are as follows: AY, 0; RY, 0; A, 4%; R, 4%; AR, 13%; RA, 7%; AH, 8%; RH, 0.

Figure 8 shows growth curves drawn using the mean values calculated for A, B and k for ad libitum-fed and restricted rats eating LB. Actual mean body weights are plotted for individual groups A, R, AR and RA at intervals from 56 to 96 weeks of age. All growth curve parameters (A, B, k) were significantly higher ($p < 0.05$) for the ad libitum-fed than for the restricted rats. Table 5 shows the analyses of variance for these parameters, and Table 6 shows the mean values. Calculated mature body size (A) of the restricted animals was 71% (372 compared with 526 g) that of the ad libitum-fed animals. The comparable comparison of terminal body weights (374 and 565 g, respectively) was 66%. Terminal body weights of rats of groups AR and RA were not significantly

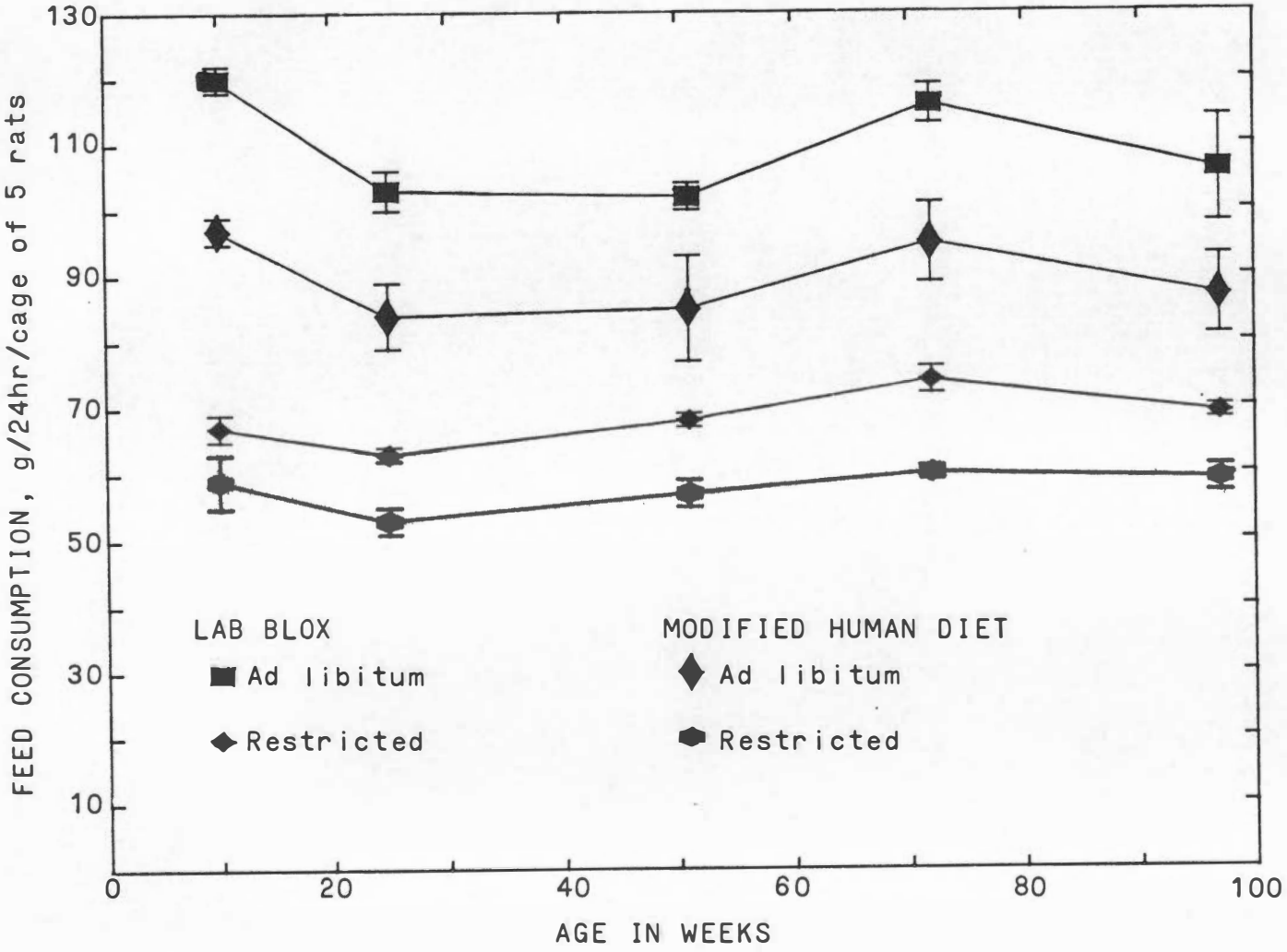


Figure 7. Influence of diet on feed intake of restricted and ad libitum-fed rats. Points show means $\pm$ SEM.

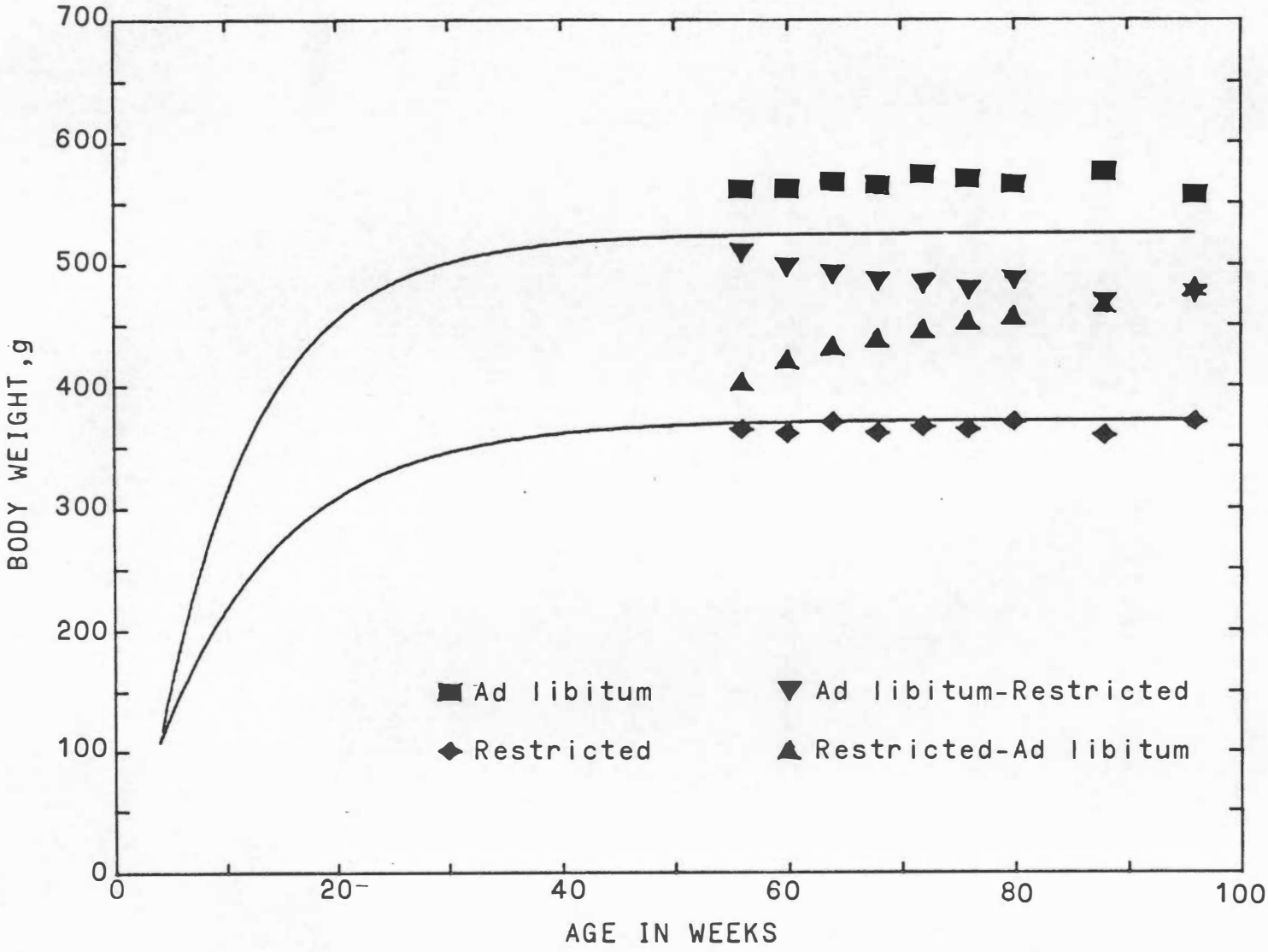


Figure 8. Influence of restriction on calculated growth curves and actual mean body weights for animals eating Lab Blox.

TABLE 5

ANALYSES OF VARIANCE OF GROWTH PARAMETERS AND TERMINAL BODY WEIGHTS

Source of Variation	df	Mean Squares			
		A	B	k	Body wt
Treatment	7	90435[1]	235886[1]	0.00189[1]	90361[1]
Rats within Treatment	87	1929	2782	0.00020	19737

[1] $p < 0.01$.

TABLE 6

INFLUENCE OF DIET AND RESTRICTION ON MEAN GROWTH CURVE PARAMETERS AND TERMINAL BODY WEIGHTS[1]

Treatment	n	Calculated Growth Curve Parameters[2] A	B	k	Actual Terminal Body Weight g
Lab Blox					
AY	12	504.8^{b}	620.5^{a}	0.1130^{a}	515^{c}
A	11	544.6^{ab}	669.4^{a}	0.1071^{ab}	565^{b}
AR	12	528.9^{ab}	650.5^{a}	0.1090^{ab}	477^{c}
Mean Ad libitum[3]	35	526.0	646.7	0.1097	
RY	12	375.5^{c}	370.7^{b}	0.0867^{c}	382^{d}
R	12	370.6^{c}	366.8^{b}	0.0798^{c}	374^{d}
RA	12	370.4^{c}	390.9^{b}	0.0994^{b}	492^{c}
Mean Restricted[3]	36	372.0	376.7	0.0886	
Modified Human Diet					
AH	12	560.6^{a}	634.7^{a}	0.0875^{c}	615^{a}
RH	12	381.0^{c}	403.2^{b}	0.0865^{c}	405^{d}
SEM for Column		± 13.3	± 18.2	± 0.0038	± 14

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

[2]Calculations based on body weight measurements during first year of life.

[3]All groups combined to form this mean were fed identically during the first year of life.

different from each other (477 and 492 g, respectively) and were 84 and 87% as great as the terminal mean body weight of animals of group A.

Growth curves for animals fed the 2 different diets on either a restricted or an ad libitum basis are shown in Figure 9. Neither ad libitum-fed nor restricted animals fed MHD had mature body weights (A) significantly different from comparable groups eating LB (see Table 6, p. 58). Mature body weight (A) of the restricted animals eating MHD was 68% (381 compared with 561 g) that of the ad libitum-fed animals. Growth rate (k) was significantly lower ($p < 0.05$) for ad libitum-fed rats eating MHD (0.0875) than for those comparably fed LB (0.1097). Growth rate was significantly lower ($p < 0.05$) for restricted (0.0886) than for ad libitum-fed animals (0.1097) eating LB. However, there was no significant difference between growth rate of restricted (0.0865) and ad libitum-fed (0.0875) rats eating MHD.

Urine volumes did not change significantly with age except for group AH which showed a significant increase ($p < 0.05$) when the mean volume of the final collection (14.46 ml) was compared with that of the first collection (8.92 ml). Therefore only urine volumes for the final collection period are shown. The analyses of variance of urinary parameters for the final collection period are shown in Table 7, and the mean values for urinary volume, osmolarity and creatinine for this period are shown in Table 8. Only the mean urine volume of group AH was significantly different ($p < 0.05$) from other means. However, volumes for groups RH, A and AR tended to be higher than for the younger and/or early-restricted (restricted from 1 month of age) rats eating LB. Osmolarity of the urine was significantly higher ($p < 0.05$) in groups AY

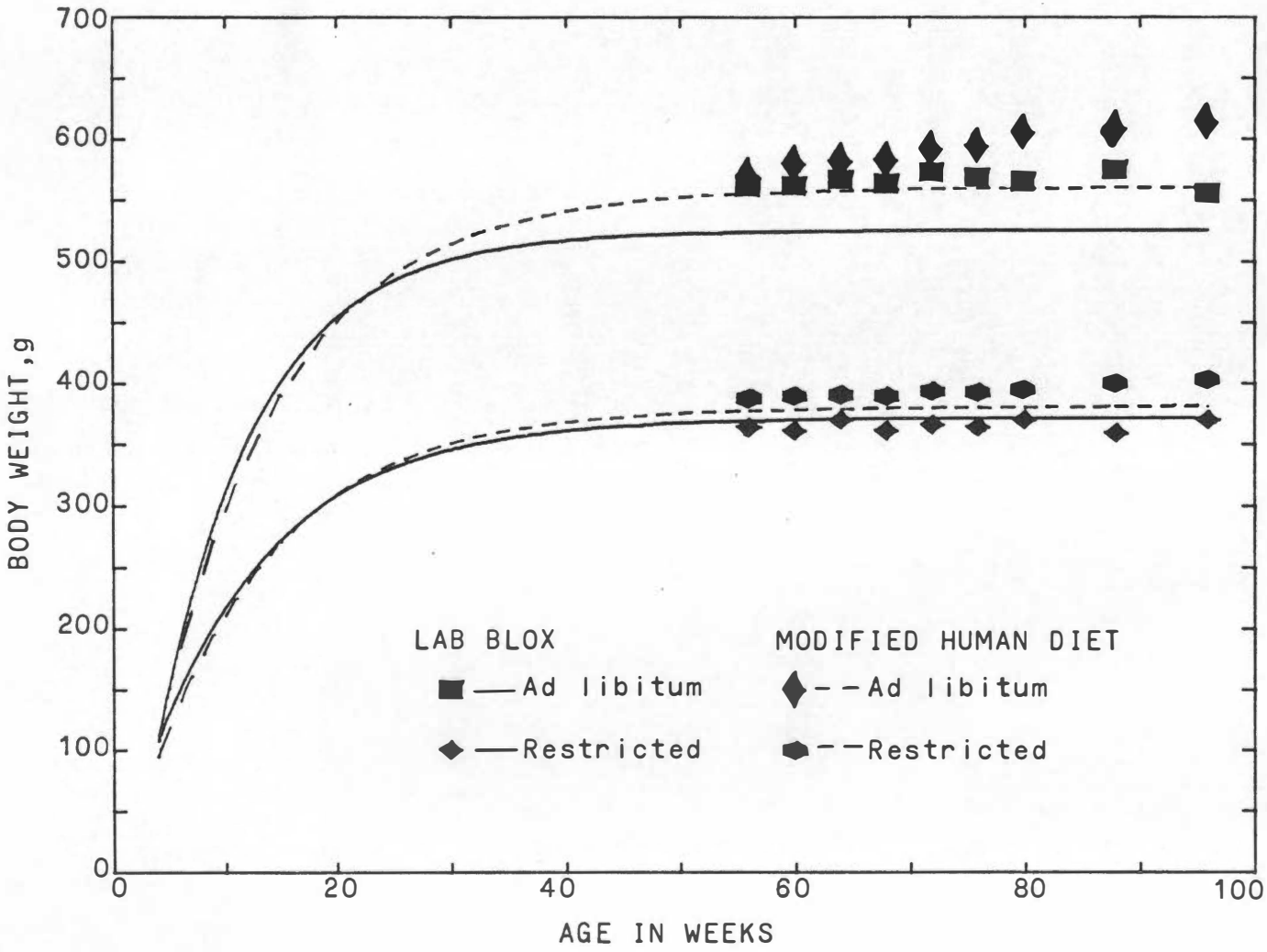


Figure 9. Influence of diet on calculated growth curves and actual mean body weights.

TABLE 7

ANALYSES OF VARIANCE OF URINARY VOLUME, OSMOLARITY AND CREATININE AT FINAL COLLECTION

Source of Variation	df	Mean Squares		
		Volume	Osmolarity	Creatinine
Treatment	7	68.91[1]	1712169[1]	102.66[1]
Rats within Treatment	87	13.41	319053[2]	3.72

[1] $p < 0.01$.

[2] 85 df for Rats within Treatment.

TABLE 8

INFLUENCE OF AGE, DIET AND RESTRICTION ON URINARY VOLUME, OSMOLARITY AND CREATININE AT FINAL COLLECTION[1]

Treatment	n	Volume ml/24 hr	Osmolarity mosmoles/liter	Creatinine mg/24 hr
AY	12	8.23[b]	2570[a]	14.12[b]
RY	12	7.38[b]	2032[b]	10.13[b]
A	11	10.33[b]	1741[b]	13.25[b]
R	12	7.42[b]	2087[b]	9.00[b]
AR	12	9.71[b]	1960[b]	12.44[b]
RA	12	7.35[b]	2706[a]	12.64[b]
AH	12	14.25[a]	1927[b]	18.80[a]
RH	12	10.57[b]	1651[b]	13.35[b]
SEM for Column		±1.01	±163	±0.50

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

and RA (2570 and 2706 mosmoles/liter, respectively) than in the other groups (Table 8) and tended to be lower for groups RH and A (1651 and 1741 mosmoles/liter, respectively) than for other groups.

As with urine volume, mean creatinine excretion only of group AH (18.80 mg) was significantly different ($p < 0.05$)--in this case higher--than other means (Table 8). Dietary restriction was associated with lower urinary creatinine levels. The analyses of variance in Table 9 show a significant ($p < 0.01$) influence of body weight on creatinine levels. Therefore, when urinary creatinine was expressed in a ratio to body weight (Table 10) much of the effect of restriction was removed. The most obvious difference shown in Table 10 is a dietary one with the ratio being consistently higher for groups fed MHD than for those eating LB. Table 10 also shows a decreased creatinine/body weight ratio with age in most groups with decreases being most pronounced for groups A and AH. Creatinine excretion, even expressed as a ratio to body weight, increased markedly in group RA when these animals were switched from restricted to ad libitum feeding. However, the drop in excretion of this group between 15 and 21 months paralleled that for groups A and AH. The drop in urinary creatinine between 15 and 21 months was significantly greater ($p < 0.05$) for groups A, RA and AH than for groups RA and RH but only the drop for group A was greater than that for R.

Urinary protein excretion was higher for ad libitum-fed than for restricted animals as early as 3 months of age. Urinary protein remained relatively constant throughout life in restricted animals eating either LB or MHD but increased dramatically as age of the ad libitum-fed animals increased. Figure 10 shows the pattern of excretion for the various

TABLE 9

ANALYSES OF VARIANCE OF URINARY CREATININE AT 5 AGES

Source of Variation	df	Mean Squares				
		3 mo	9 mo	12 mo	15 mo	21 mo
Treatment	5	102.68[1]	181.96[1]	78.68[1]	173.59[1]	125.41[1]
Body weight	1	121.91[1]	113.29[1]	116.41[1]	59.08[1]	38.94[1]
Rats within Treatment	65	2.34	1.63	4.55	3.00	3.12

[1] $p < 0.01$.

TABLE 10

INFLUENCE OF DIET AND RESTRICTION ON URINARY CREATININE/ BODY WEIGHT RATIO AT 5 AGES[1]

		mg Creatinine/100 g Body Wt/24 Hr					
Treatment	n	3 mo	9 mo	12 mo	15 mo	21 mo	Decrease[2] 15 mo-21 mo
Lab Blox							
Ad libitum	24	3.11^{c}	2.70^{c}				
A	12			2.56^{c}	2.80^{c}	2.33^{c}	0.46^{ab}
AR	12			2.77^{bc}	2.67^{c}	2.63^{bc}	0.06^{c}
Restricted	24	2.80^{b}	2.58^{c}				
R	12			3.17^{ab}	2.69^{c}	2.45^{bc}	0.24^{bc}
RA	12			3.26^{ab}	3.25^{b}	2.68^{bc}	0.57^{a}
Modified Human Diet							
AH	12	3.69^{a}	3.74^{a}	3.35^{a}	3.72^{a}	3.20^{a}	0.51^{ab}
RH	12	3.63^{a}	3.50^{b}	3.30^{ab}	3.30^{b}	3.22^{a}	-0.02^{c}
SEM for Column		± 0.07	± 0.06	± 0.07	± 0.06	± 0.06	± 0.09

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

[2]Decrease is the mean of differences for individual rats.

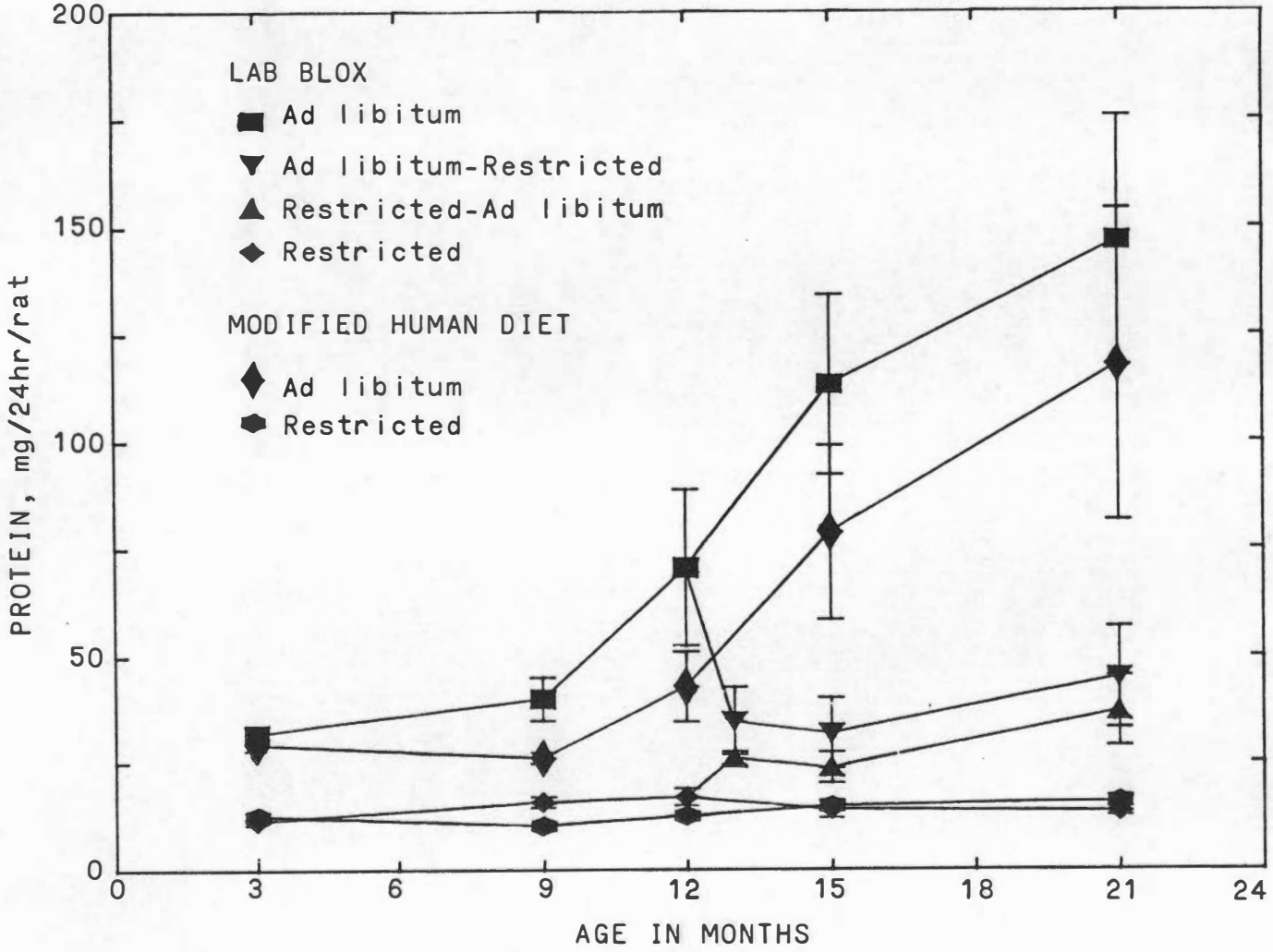


Figure 10. Influence of diet and restriction on proteinuria in rats 3 to 21 months of age. Points show means ± SEM.

feeding regimens from 3 to 21 months of age. Urinary protein at 21 months was 4 1/2 times as high as the 3 month level for ad libitum-fed animals eating LB and 4 times as high for those eating MHD. Protein excretions of groups AR and RA were similar to each other at 15 and 21 months of age, and though slightly higher than for groups R and RH, were much lower than those for animals always eating ad libitum. In addition to differences in means for protein excretion from 1 group to another, there were differences in variances making it impossible to compare statistically the different populations. Means, standard errors and ranges for the 6 treatments for the final collection period are shown in Table 11. As can be seen, there are very small ranges and standard errors for restricted animals and extremely large ones for old ad libitum-fed rats. At 21 months, ranges varied from the 9.67-20.54 mg of group R to the 17.06-466.12 mg of group AH.

Rank correlations showed urinary protein to be positively and significantly correlated ($r = 0.84$; $p < 0.05$) with scores for kidney lesions observed microscopically in kidneys from animals of groups A and AH. None of the animals experiencing feed restriction from 1 month of age (RY, R, RA and RH) showed any microscopic abnormalities other than glomerular thickening. Only 1 rat (8.3%) of group AY and 2 rats (16.6%) of group AR showed other changes. Two of these were moderate inflammation and 1 (group AR) was metaplasia and calcium deposits of the kidney pelvis, a condition which did not seem to affect proteinuria, while the occurrence of casts, chronic inflammation and cysts was quite common in kidneys from rats of groups A and AH. In the 2 ad libitum-fed groups, 16% of animals received lesion scores of 2; 16% were scored 3;

TABLE 11

INFLUENCE OF DIET AND RESTRICTION ON URINARY PROTEIN AT FINAL COLLECTION

Treatment	n	Protein mg/24 hr	Protein Range	
			Low mg/24 hr	High mg/24 hr
A	12	146.81 ± 28.92[1]	14.33	342.62
R	12	13.54 ± 0.98	9.67	20.54
AR	12	45.00 ± 11.77	15.04	144.74
RA	12	37.10 ± 8.19	14.40	89.91
AH	12	117.82 ± 36.36	17.06	466.12
RH	12	15.87 ± 1.27	8.94	23.50

[1]Mean ± SEM.

12% were scored 4; and 12%, scored 5. The lesion scores did not account for all the differences in protein excretion since the urinary proteins for animals receiving scores of 1 were different in both means and variances in ad libitum-fed animals eating either diet when compared with animals restricted throughout life receiving the same lesion score (Table 12).

Blood urea nitrogen (BUN) level at sacrifice (Tables 13, 14) was significantly ($p < 0.05$) lower for the 2 groups eating MHD (AH, 12.0 mg; RH, 12.7 mg) than for those eating LB and tended to be higher for animals of group A (26.7 mg) than for other groups eating the same diet (average, 16.2 mg). None of the serum creatinine values were significantly different from each other. However, values for old restricted rats fed LB (R, RA and AR) tended to be lower than for other groups.

Analyses of variance for kidney weights and percent protein are shown in Table 15 and means are shown in Table 16. Total kidney weight was greatly influenced by restriction with the smaller kidneys coming from rats of groups RY, R and RH (RY, 2.172; R, 2.373; and RH, 2.579 g). Kidneys from groups AR (3.068 g) and RA (3.013 g) were not significantly different from each other nor was the difference between weights of kidneys from groups AH (4.167 g) and A (3.700 g) significant. When total kidney weight was expressed as percent of body weight, restriction differences became less apparent and age differences more so with kidneys from old ad libitum-fed rats being a significantly ($p < 0.05$) higher percentage of total body weight than were kidneys from younger animals. Total kidney weights expressed as percentages of body weights of old restricted animals (R, AR, RA, RH) were not significantly different

TABLE 12

MEAN PROTEIN EXCRETION VALUES BY TREATMENT
FOR RATS WITH LOWEST LESION SCORE

Treatment	n	Protein mg/24 hr	Protein Range Low mg/24 hr	Protein Range High mg/24 hr
AY	11	39.64 ± 8.22[1]	8.14	85.22
RY	12	13.69 ± 0.99	10.20	20.83
A	4	56.24 ± 16.40	14.33	93.15
R	12	13.54 ± 0.98	9.67	20.54
AR	10	36.96 ± 9.04	15.04	109.26
RA	12	37.10 ± 8.20	14.44	89.91
AH	6	50.54 ± 13.03	17.06	104.31
RH	12	15.87 ± 1.27	8.94	23.50

[1]Mean ± SEM.

TABLE 13

ANALYSES OF VARIANCE OF BLOOD PARAMETERS

Source of Variation	Creatinine		Blood Urea Nitrogen	
	df	Mean Square	df	Mean Square
Treatment	7	7.002[1]	5	366.30[2]
Rats within Treatment	87	3.135	65	66.24

[1] Not significant.

[2] $p < 0.01$.

TABLE 14

INFLUENCE OF AGE, DIET AND RESTRICTION ON LEVELS OF BLOOD UREA NITROGEN AND SERUM CREATININE[1]

Treatment	n	Blood Urea Nitrogen mg/100 ml	Serum Creatinine µg/ml
AY	12	--	3.64[a]
RY	12	--	4.79[a]
A	11	26.7[a]	3.80[a]
R	12	17.1[a]	2.97[a]
AR	12	15.1[a]	2.76[a]
RA	12	16.5[a]	2.85[a]
AH	12	12.0[b]	3.97[a]
RH	12	12.7[b]	4.51[a]
SEM for Column		±2.0	±0.52

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

TABLE 15

ANALYSES OF VARIANCE OF TOTAL KIDNEY WEIGHT, PERCENT PROTEIN AND TOTAL KIDNEY WEIGHT/BODY WEIGHT RATIO

Source of Variation	df	Mean Squares		
		Kidney wt	Percent Protein	Kidney wt/ Body wt
Treatment	7	4.20[1]	5.41[1]	0.0000012[2]
Rats within Treatment	82	0.24	0.91	0.0000006

[1] $p < 0.01$.

[2] $p < 0.06$.

TABLE 16

INFLUENCE OF AGE, DIET AND RESTRICTION ON KIDNEY WEIGHT, PERCENT PROTEIN AND RATIO OF TOTAL KIDNEY WEIGHT/ 100 G BODY WEIGHT[1]

Treatment	n	Total Kidney wt (Right + Left) g	Protein Percent	Total Kidney wt/ 100 g Body wt
AY	12	2.999^{bc}	12.6^{bc}	0.58^{b}
RY	12	2.172^{c}	13.3^{ab}	0.57^{b}
A	11	3.700^{a}	12.3^{c}	0.67^{a}
R	12	2.373^{d}	13.8^{a}	0.64^{ab}
AR	12	3.068^{b}	13.2^{ab}	0.64^{ab}
RA	12	3.013^{bc}	13.6^{a}	0.61^{ab}
AH	12	4.167^{a}	12.1^{c}	0.68^{a}
RH	12	2.579^{cd}	13.8^{a}	0.64^{ab}
SEM for Column		± 0.140	± 0.3	± 0.02

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

from those of either young rats or old ad libitum-fed animals. Kidney protein expressed as a percentage of kidney wet weight from rats restricted at any time and fed either diet was significantly ($p < 0.05$) higher than that of kidneys from old rats fed either diet ad libitum.

The analyses of variance for PAH transport are shown in Table 17, and the mean values are in Table 18. PAH transport (expressed as μg PAH/mg kidney protein) was significantly ($p < 0.05$) higher by kidney slices from young ad libitum-fed rats (1.031) than by kidney slices from old ad libitum-fed ones eating LB (0.598) but not significantly higher than for old animals that had been restricted at any time (R, 0.873; AR, 0.868; RA, 0.907). Transport by kidney slices from young restricted rats tended to be higher than that by young ad libitum-fed rats and was significantly higher ($p < 0.05$) than that by slices from old ad libitum-fed animals. PAH transport by kidney slices from restricted animals eating MHD (1.181) was significantly higher ($p < 0.05$) than that by ad libitum-fed rats of the same age eating LB (0.598) or the same diet (0.882). Transport tended to be higher by kidney slices from animals eating MHD ad libitum than by those from rats comparably treated but eating LB.

The same general pattern was observed when total renal transport of PAH per 100 g body weight was calculated (called total PAH). Restriction differences became more apparent, and age and diet differences, slightly less so (see Table 18). Total PAH transport was significantly lower ($p < 0.05$) by kidneys from animals of group A (0.470) than for those of either age or eating either diet which had been restricted at any time (RY, R, AR, RA, RH). Total PAH transport was

TABLE 17

ANALYSES OF VARIANCE OF PAH TRANSPORT

Source of Variation	df	Mean Squares	
		PAH/Protein	Total PAH/ Body Wt
Treatment	7	0.4480[1]	0.3484[1]
Rats within Treatment	83	0.1012	0.0624

[1] $p < 0.01$.

TABLE 18

INFLUENCE OF AGE, DIET AND RESTRICTION ON PAH TRANSPORT BY KIDNEY SLICES[1]

Treatment	n	PAH μg/mg Protein	Total PAH μg/100 g Body Wt
AY	12	1.031^{abc}	0.752^{bc}
RY	12	1.240^{a}	0.936^{ab}
A	10	0.598^{d}	0.470^{d}
R	11	0.873^{cd}	0.781^{bc}
AR	12	0.868^{cd}	0.736^{bc}
RA	12	0.907^{c}	0.753^{bc}
AH	10	0.882^{cd}	0.688^{cd}
RH	11	1.181^{ab}	1.090^{a}
SEM for Column		± 0.093	± 0.072

[1]Means in a column sharing a common superscript are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range Test.

significantly higher ($p < 0.05$) by kidneys from rats of group RH (1.090) than by those from any other group except RY (0.936), and total transport tended to be better by kidneys from rats eating MHD ad libitum than by those comparably fed LB.

Na-K-ATPase activities (expressed as percents of total ATPase activities) in kidney microsomes of the various groups were not significantly different from each other. However, the percentage of Na-K-ATPase activity tended to be higher in young than in old ad libitum-fed animals (24% compared with 18%) and tended to be lower in animals eating MHD (16%) than in those comparably fed LB. There was a slight tendency toward a higher percentage Na-K-ATPase activity in old restricted animals (22%) than in ad libitum-fed ones (18%) eating LB.

CHAPTER V

DISCUSSION

The levels of feed restriction employed in this study were similar to those used by Berg and Simms (17,18) and Nolen (11) in studies with rats and followed the same feeding pattern described by Halai (23) for restriction of mice. Levels of restriction were less severe than those generally employed by Ross (12,13,14) or Barrows and coworkers (20,21,22). The intermittent type restriction used in this study permitted some variation in intake depending on individual differences in appetite--which might be assumed to be related to need for food. This type restriction also allowed all rats within a cage to eat as much as they wanted within the allotted time period rather than permitting dominant rats within a cage to eat more than their share thus further restricting the less dominant animals. In the present study, rats restricted after 12 months of age had greater feed intakes than did those of the same age restricted from 1 month since the animals which had previously been fed ad libitum had larger stomach capacities and could eat a larger amount in the length of time feed was available to them.

It is important to note that the type and levels of restriction employed in this study were not severe enough to cause a high mortality rate during the first year such as described by Widdowson and coworkers (7) nor did restriction of the 12-month-old animals put such a stress on the system as to cause a high death rate in this group such as was reported by Ross (12) when he restricted mature rats to an intake of 6-8 g per day. In fact, none of the 50 animals restricted from 1 month of

age died before 23 months, and only 1 (2%) died a natural death between then and the conclusion of the study when rats were 25 months of age. Only 1 rat (4%) of group A died before the conclusion of the study, and although 2 rats (8%) of group AH died, both of these deaths occurred between 23 and 25 months of age. Deaths in group AR and RA were 4 (13%) and 2 (7%) respectively, and seemed to be totally unrelated to switching from ad libitum to restricted feeding or vice versa.

Although it is impossible to know certainly based on the data from this study, it seems reasonable to suppose that weight gains of ad libitum-fed animals after 1 year of age were due primarily to deposits of adipose tissue. This hypothesis is supported by comparison of growth curves based on actual body weights to 1 year and projected for the second year of life with actual mean body weights for the second year. The actual mean body weights for ad libitum-fed animals fell considerably above the curve while those for restricted animals fell directly on the curve. It might be further hypothesized that the loss of body weight after restriction was initiated at 12 months of age (group AR) was due primarily to loss of adipose tissue and that such restriction, which might be considered only a "weight control" measure, has beneficial effects in retarding the development of kidney lesions and delaying the deterioration of kidney function. This is contrary to previous suggestions by other investigators (12,21) that if restriction is to have any beneficial effects it must be begun in early life.

Although growth rate (k) did not correlate significantly with kidney function of animals within any individual treatment group, there <u>were</u> slower growth rates and also, in general, healthier kidneys in

restricted than in ad libitum-fed rats eating LB. That the 2 conditions might be related is further indicated by the slower growth rate of animals eating MHD ad libitum compared with that of similarly treated animals eating LB, a situation accompanied by a decided tendency toward better kidney transport of PAH and lower incidence of lesions, as indicated by both lesion score and degree of proteinuria.

Another possible explanation for the tendency toward better kidney function for animals eating MHD compared with those eating LB is a difference in protein intake. Although percent protein in the diets was very similar (24.5% for LB and 21.5% for MHD) caloric value was quite different (2.75 calories/g for LB and 4.11 calories/g for MHD). The lower feed intake (g/day) for groups eating MHD resulted in protein intakes 30-60% higher for LB groups than for animals similarly fed MHD. For example, at 10 weeks of age animals fed LB ad libitum were eating 120 g/day/cage of 5 rats while a comparable figure for rats fed MHD was 97 g which gave them protein intakes of 29.4 and 20.9 g/day/cage of 5 rats, respectively. The caloric level of MHD was similar to the level recommended by the National Research Council (61) for growing rats, and the protein percentage was considerably higher (21.5% compared with 13.3%). Since protein intake was higher still for rats eating LB ad libitum, and since high protein intake has been reported to be detrimental to kidney function (77), the high protein consumption of LB groups might have been related to the renal damage observed. It is also possible that the elevated protein intakes of groups eating LB ad libitum might have been responsible for the accelerated growth in these animals as indicated by their higher k values as compared to those of group AH.

The absence in rats restricted from 1 month of age of kidney lesions which could be identified microscopically agrees with the work of Berg and Simms (18). However, they reported a 100% incidence of lesions in unrestricted male rats at 747 days, while in the present study there were 4 rats (33%) fed LB ad libitum and 6 rats (50%) fed MHD ad libitum which did not have detectable lesions (other than glomerular thickening and possibly a small number of casts) by approximately the same age. It is noteworthy that only 1 rat (8%) in the group fed ad libitum to 12 months before restriction was begun (AR) showed evidence of inflammation, and this was only moderate.

Since there were treatment differences in proteinuria even when values from rats receiving lesion scores of 1 were compared, it seems that quantitative determination of urinary protein must detect renal lesions before they can be easily identified and quantified by microscopic examination of kidney tissue. There are factors present which influence lesion score and not urinary protein levels (i.e., calcium deposits of kidney pelvis were scored 2 but did not influence protein excretion), but obviously the measurements are highly related as indicated by their correlation coefficient ($r = 0.84$). Therefore it seems that urinary protein level, which can be easily determined without having to sacrifice the animal, can be used as an indication of renal lesions in the rat. The increased proteinuria with age is consistent with both the work of Everitt (45) and that of Beauchene and coworkers (46). However, it should be noted that dietary manipulation altered the occurrence and/or severity of lesions thus seeming to slow the rate of the kidney aging process.

Although in the present study a significant age-associated increase in urine volume was shown only for group AH, a tendency toward an increase in this parameter with age was seen for old ad libitum-fed animals eating LB. The tendency toward lower urine volume for restricted than for ad libitum-fed animals at 21 months of age might therefore be considered a beneficial effect of restriction. The increase in urine volume reported by Everitt (44) occurred between 750 and 953 days. Since no collections in the present study were obtained from animals as old as 750 days, this probably explains why some of the present increases in urine volume did not obtain significance.

The high osmolarity of urine from young ad libitum-fed rats compared with values for older animals is consistent with the findings of Dicker and Nunn (43) that concentrating ability of the kidneys decreased with age. The high osmolarity of urine from group RA would appear to indicate a beneficial effect of dietary treatment on this parameter. Probably the long periods of time between feeding in the type restriction used in the present investigation is related to the failure of other restricted rats (RY, R, AR and RH) to show beneficial change in osmolarity. For example, with feed restriction and its consequential reduction in solute load, there was perhaps less need for production of a concentrated urine. Thus osmolarity may have reflected feeding pattern rather than kidney function for these groups. Based on findings with group RA, it is possible that if other restricted animals were fed on an ad libitum basis for a short time, they too might show a higher urinary osmolarity, a condition more consistent with the other indications of relatively good kidney function by animals of these groups.

The findings of a decreased transport of PAH by kidney slices from ad libitum-fed old rats as compared with younger animals fed the same diet is in agreement with findings by Adams and Barrows (36) and Beauchene et al. (37). The improved transport by kidneys from rats restricted for any of the time intervals used in the present investigation is another indication that restriction has contributed to making these animals more like chronologically younger ones. The fact that restriction beginning at 12 months of age resulted in slightly better PAH transport (when expressed as PAH/mg protein) than that by kidneys from rats restricted from 1 month seems especially important since it indicates that "weight control" in an adult can be beneficial in delaying kidney changes which would otherwise occur with age. The improved PAH transport by kidney slices from rats fed MHD as compared with those comparably fed LB may again relate to the adverse effects of high intakes of dietary protein. Since percent protein was higher in kidneys of restricted than of ad libitum-fed rats, it follows that expressing PAH on a wet-weight basis of renal tissue, as reported by other investigators (36,37), would have shown even greater differences than were reported here where values were based on renal tissue protein.

As might be expected, kidneys were larger in ad libitum-fed than restricted animals. However, most of these differences were removed when kidney weight was expressed as a percent of body weight. This agrees with the report by Lee and Lucia (78) that restriction does not change the kidney weight/body weight ratio of young rats. The slightly higher ratios for groups fed ad libitum throughout life could be related

to some renal hypertrophy or even to fluid retention by the kidneys as their function began to deteriorate.

The present study failed to confirm the correlation between Na-K-ATPase activity and PAH transport which was reported by Beauchene et al. (37). However, there was a tendency toward decreased activity of the enzyme with age which was reported by the earlier study. The tendency toward a lower level of Na-K-ATPase activity of microsomes accompanied by a higher level of PAH transport by kidney slices from rats fed MHD seems to support the idea suggested by Hendler et al. (53) and by Whittembury (54) that a second transport mechanism, one which is Na-K-ATPase independent, also may be operating.

Values for urinary and serum creatinine and for BUN seemed to be more diet- than restriction-related. BUN tended to be higher for rats eating LB ad libitum than for any other group, a finding consistent with the poor kidney function evidenced by low PAH transport by kidneys from this group. The low BUN and high PAH transport by animals eating MHD are both consistent with the high level of kidney function observed in these animals. The diet-related difference in serum creatinine would not seem to be an indication of retention rather than excretion of creatinine by groups eating MHD--especially since these groups also had higher levels of urinary creatinine than comparably fed LB groups. The high serum and urinary creatinine levels associated with MHD would seem to indicate a difference in dietary intake of creatinine or a difference in metabolism, possibly increased creatinine synthesis, of animals fed this diet. The decreased urinary creatinine between the ages of 15 and 21 months for ad libitum-fed rats eating either diet is consistent

with the findings of Everitt (45). The smaller change in urinary creatinine of restricted rats between these ages seems to be another indication of beneficial effects of restriction. Serum creatinine values did not seem to relate to other measures of kidney function except in cases of severely impaired kidney function (as indicated by such factors as extremely elevated BUN and extremely low PAH transport) in which cases creatinine levels were also abnormal (high).

It can be concluded that feed restriction during either the first, the second or both years of the life of a rat was beneficial in delaying age associated changes in kidney function as measured by PAH transport, proteinuria and kidney lesions. BUN levels and serum and urinary creatinine levels also tended to be influenced favorably by restriction. Most parameters were modified by diet as well as by restriction with kidney performance being generally improved in animals eating MHD as compared with those comparably fed LB.

CHAPTER VI

SUMMARY

Kidney function of 12- and 24-month-old male rats fed ad libitum either a commercial feed (LB) or a modified human diet (MHD) was compared with that of rats of the same age and eating the same diet but on a restricted basis. When restriction was used, it was continued for one of the following intervals: 1 month to 24 months, 12 months to 24 months, or 1 month to 12 months of age. Restriction was accomplished by making feed available to animals for only 15 out of each 48 hours after a 1-month adaptation period.

Body weights, growth curve parameters and feed intakes were significantly lower for restricted than for ad libitum-fed rats eating either diet with the single exception that growth rate (k) was not different between animals eating the modified human diet on an ad libitum and on a restricted basis. Body weights for restricted animals 12-24 months of age fell directly on growth curves extrapolated from those calculated using body weights for the first year. However body weights for 12-24 month-old ad libitum-fed animals fell above their extrapolated curves indicating an accumulation of adipose tissue during the second year in these animals.

Urinary data from collections obtained at 3, 9, 12, 15 and 21 months of age showed an increased volume with increasing age of rats only for ad libitum-fed animals eating the modified human diet. Urine volume for this group was significantly higher at 21 months than those for other rats of the same age. Volumes for 21-month-old restricted

animals eating modified human diet and for ad libitum-fed rats of the same age eating commercial feed tended to be higher than for younger and/or restricted rats eating LB. Urine osmolarity for the final collection period was highest for animals eating LB on a restricted basis the first year and ad libitum the second. Osmolarity also was significantly higher for 12-month-old ad libitum-fed rats than for older animals.

Urinary creatinine levels increased with increasing body weights and were significantly higher for groups eating MHD than for those eating LB. There was a greater reduction with age in creatinine excretion (expressed as a ratio to body weight) for rats fed ad libitum either from 12 months or throughout the study than for those that were restricted throughout or during the second year.

Protein excretion was much higher and the variance was much greater for ad libitum-fed than for restricted groups with differences becoming more pronounced as the age of the animals increased. Urinary proteins for groups restricted for either the first or the second year only were similar to each other and much lower than for ad libitum-fed groups. The incidence and severity of microscopic kidney lesions were correlated positively with protein excretion of individual animals of ad libitum-fed groups, but there were treatment differences in urinary protein of restricted animals which could not be accounted for by microscopic observation.

Blood urea nitrogen (BUN) values were significantly lower for groups eating MHD than for those eating LB, and BUN tended to be higher for ad libitum-fed animals eating LB than for those animals eating a

restricted level of the same diet. However serum creatinine levels tended to be higher for groups fed MHD than for those eating LB.

Transport of PAH by kidney slices was higher for young than for old animals eating LB ad libitum, but was not significantly different when young ad libitum-fed rats were compared with old animals that had been restricted at any of the time intervals employed in the study. There was a tendency for transport of PAH to be higher by kidneys from rats eating MHD than by those comparably fed and of the same age eating LB. Total PAH transport calculated on the basis of total kidney protein and divided by body weight was higher by kidneys from rats restricted at any time during their lives than by those which were ad libitum-fed.

Activity of Na-K-ATPase (expressed as a percent of total ATPase activity) was not significantly different when the different groups were compared. However, there was a tendency for activity to be higher for young than for old rats and lower for those groups eating MHD than for ones eating LB.

In general, the hypotheses were supported and kidney function, as measured by the tests of this study, was better in restricted than in ad libitum-fed animals. Some parameters were modified more by diet than by restriction with kidney performance being, in most cases, at a higher level in animals eating MHD as compared with those comparably fed LB. Differences in kidney function among treatment groups may have been related to growth rates as well as intakes of calories and/or protein.

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LITERATURE CITED

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VITA

Sarah May Tucker was born in Livingston, Tennessee, where she later attended Livingston Elementary School and Livingston Academy. In May, 1968, she was graduated Magna Cum Laude from Carson-Newman College, Jefferson City, Tennessee, with the degree of Bachelor of Science in Home Economics. She began graduate work at The University of Tennessee in the summer of that year and served as a graduate teaching assistant in the Department of Nutrition during the academic year 1968-1969. After receiving the Master of Science degree in August, 1969, she was an Instructor in the Division of Home Economics at Georgia Southern College, Statesboro, Georgia, for two years. In the summer of 1971, she returned to The University of Tennessee to work toward the Ph.D. degree, which was granted in August, 1974. After receipt of the Ph.D. degree with a major in nutrition, she went to Indiana State University, Terre Haute, Indiana, as an Assistant Professor in Home Economics.